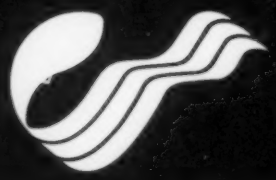

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Significance of Egg and Larval Size to Recruitment Variability of Temperate Marine Fish

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Pepin, P., and R. A. Myers. 1991. Significance of egg and larval size to recruitment variability of temperate marine fish. Can. J. Fish. Aquat. Sci. 48: 1820–1828.

Recruitment variability is commonly associated with fluctuations in abundance of marine fish populations. Previous studies have focussed on stock-specific correlative or mechanistic models or on comparisons of recruitment variations of several stocks or species. The purpose of this study is to determine whether recruitment variability of commercial marine fish populations is associated with either size or the duration of early life history stages. The analysis was performed with data from 86 stocks representing 21 species of commercial marine fish. Univariate analysis shows that neither egg size nor the length at hatch is significantly correlated with recruitment variability. The change in length during the larval phase, which is representative of the duration of the stage, is significantly positively correlated with recruitment variability. Multivariate analysis shows that recruitment variability increases with increasing length at metamorphosis but that recruitment variability is poorly associated with length at hatch. The degree of serial correlation is related to the relative duration of egg and larval stages. The results clearly indicate that recruitment variability is linked to characteristics of early life history stages.

La variabilité du recrutement dans les populations de poissons marins est souvent liée aux fluctuations de l'abondance. Les études faites jusqu'ici se sont articulées autour de modèles mécanistiques ou corrélatifs, caractéristiques d'un stock, ou autour de la comparaison des variations de recrutement de plusieurs stocks ou de plusieurs espèces. Dans l'étude présentée ici, nous visions à déterminer si la variabilité du recrutement dans des populations de poissons marins d'intérêt commercial est liée à la taille des poissons ou à la durée des premiers stades de leur développement. Pour l'analyse, nous avons employé des données concernant 21 espèces de poissons marins d'intérêt commercial réparties entre 86 stocks. L'analyse univariée révèle que ni la taille des oeufs, ni la longueur du poisson à l'éclosion ne sont en corrélation significative avec la variabilité du recrutement. Par contre, le changement de longueur durant la phase larvaire, changement représentatif de la durée du stade, est en corrélation positive significative avec la variabilité du recrutement. L'analyse multivariée révèle que la variabilité du recrutement augmente en fonction de la durée de la métamorphose, mais n'est que faiblement liée à la longueur à l'éclosion. Le degré de corrélation sériale est lié à la durée relative de l'incubation et du stade larvaire. Les résultats montrent clairement que la variabilité du recrutement est liée à des caractéristiques des premiers stades du développement.

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Recruitment variability is an important factor contributing to fluctuations in the abundance of commercial marine fish populations (Jones 1983). Research into the causes of the variability has focussed on stock-specific correlative (e.g. Sutcliffe et al. 1983) or mechanistic models (e.g. Leggett et al. 1984) or on comparison of recruitment fluctuations of several species (Garrod and Colebrook 1978; Koslow 1984; Shepherd et al. 1984) or stocks of taxonomically similar species (Koslow et al. 1987). The general conclusions are that within a given system or region, species or stocks with similar life histories exhibit similar fluctuations in recruitment. However, there have been no systematic comparisons of life history characteristics and recruitment variability (although see Rothschild and DiNardo 1987).

Fluctuations in year-class strength represent fluctuations in the reproductive potential of a population. Any life history feature affecting reproductive potential is subject to natural selection (Cole 1954). Hjort (1914) proposed that year-class strength of marine fish populations was determined during the early life history (i.e. egg and larval stages). Hjort (1914) and others (May 1974; Houde 1987; Beyer 1989) argued that because of the high development, growth, and mortality rates of fish eggs and larvae, slight variations in these vital rates could result in substantial variations in survival and hence recruitment. Thus, any feature(s) influencing early life history survival can be subject to selection. Smith and Fretwell (1974) and Stearns (1980, 1983) proposed that the trade-off between numbers and size of offspring is an important factor determining reproductive potential. It has been proposed that increasing offspring size in fish is likely to decrease their vulnerability to environmental perturbations through reduced susceptibility to starvation, greater foraging ability at yolk absorption (Blaxter and Hempel 1963; Miller et al. 1988), and reduced risk of predation (Pepin et al. 1987; Miller et al. 1988). Reviews by Peterson and Wroblewski (1984), McGurk (1986), Houde (1989), and Pepin (1991) indicate that the instantaneous mortality rates of fish eggs and larvae are high and that variability increases as the daily growth and mortality rates increase. One might expect that reducing offspring size will lead to more variable year-class strength (Hjort 1914; May 1974; Miller et al. 1988). However, Smith (1985), Houde (1987), Beyer (1989), and Pepin (1991) argued that it is variability in the cumulative mortality over a

stage or size interval that may be significant in determining variations in year-class strength. In fact, the stage over which cumulative mortality is most variable is likely to be the stage over which recruitment variability is determined. Cumulative mortality will be influenced not only by the magnitude and variability in daily mortality rates but also by the daily development and growth rates as well as the duration of the stage or the size interval over which they are acting. Consider that cumulative mortality (Z) of a population from one stage or size (a) to another (b) is a direct function of the instantaneous growth ($g[x]$) and mortality ($\mu[x]$) such that

$$(1)\quad Z = \int_a^b \frac{\mu[x]}{g[x]}\,dx$$

where x are the factors that influence the vital rates. The survivorship is

$$(2)\quad \ell\,(a, b) = \frac{N_b}{N_a} = \exp(-Z)$$

where N_i are the number of individuals at stage i. Parameterizing equation 1 using general size-dependent relationships from Pepin's (1991) review (see Table 1 for details) indicates that (1) slight variations in either $g[x]$ or $\mu[x]$ can lead to substantial variations in survivorship (Table 1) and (2) increasing the duration of a stage or the length interval over which cumulative mortality is measured leads to increasing variations in survivorship for a given percentage variation in vital rates (Table 1), as shown by Smith (1985), Houde (1987), and Beyer (1989). If recruitment variability is determined by the magnitude and variability in instantaneous vital rates, we would expect that decreasing offspring size will lead to increasing variations in recruitment. However, if recruitment variability is determined by the magnitude and variability in cumulative mortality rates, we would expect that increasing the duration of a stage or the change in length during that stage will lead to increasing variations in recruitment.

The purpose of this study is to ascertain whether variations in recruitment of commercial marine fish populations are related to the size or change in size during their egg and/or larval stages. We restrict our analysis principally to species living in temperate marine environments because this reduces possible bias due to differences in habitat. The analysis is confined to investigating differences between species rather than between stocks of the same species. Although there is evidence that egg size varies within species and environments (Blaxter and Hempel 1963; Bagenal 1971; Ware 1977; Markle and Frost 1985), there are insufficient data to examine these relationships further. Data on recruitment variability of commercial marine fish populations provide a unique opportunity for study because recruitment is measured after major variations in survival have occurred (excluding fishing mortality).

In this study, we will establish that recruitment variability is representative of variations in survival before assessing the association (correlation) between recruitment variability and the size of eggs, hatchlings, larvae, and the change in length during the larval phase, as a measure of the duration of this stage.

Data Sources and Analysis

Recruitment and Survival

To establish that recruitment is a good measure of variations in survival, we will first show that there is a significant relationship between variations in recruitment and variations in survival. Data on recruitment and survival were obtained from Garrod's (1982) summary for 12 commercial marine fish stocks. Garrod calculated each population's egg production based on their distributions of numbers, weights, and weight-dependent fecundity. All data were estimated in trillions of eggs produced (10^{12}) and millions of recruits (10^6) (age 1+). For each stock, the standard deviation of the log-transformed recruitment (log R_t) and survival (log (R_t/E_t), where E_t is the number of eggs produced by the stock) was estimated. The relationships between recruitment variability and survival was estimated using regression analysis.

Recruitment Variability and Size

Most recruitment time series were obtained from Myers et al.'s (1990) summary for stocks under the jurisdiction of the North Atlantic Fisheries Organization (NAFO) and the International Council for the Exploration of the Sea (ICES). Other data were obtained from Butterworth (1983), Crawford et al. (1983), and MacCall (1983). Recruitment data from 86 stocks representing 21 species (Table 2) were derived from sequential population analysis (SPA). The age at recruitment was consid-

TABLE 1. Survivorship and ratio in survivorship at metamorphosis due to changes (±2 to ±10%) in either daily growth or daily mortality rates in relation to changes in length during the larval phase. Calculations are done by substituting $g[L] = 0.06L^{0.50}$ and $\mu[L] = 0.50L^{-0.50}$, where L is length (mm), into equation 1. Formulas for $g[L]$ and $\mu[L]$ are approximations based on Pepin's (1991) review and are consistent with general principles of size-dependent growth and mortality rates of larval fish. Temperature was assumed to be 10°C. Perturbations in vital rates are calculated as $g^* = cg$ and $\mu^* = f\mu$, where c and f are constants representing 1 ± the percentge change in vital rates. Survivorship is estimated using equation 2. Ratios listed below are approximations, since equal but opposite changes in vital rates will not result in equal changes in survivorship.

Length at hatch, L_0 (mm)	Length at metamorphosis, L_m (mm)	Survivorship	Ratio of survivorship due to a percentage change in vital rates		
			±2%	±5%	±10%
5	8	0.0199	1.17	1.48	2.19
5	11	0.00140	1.30	2.92	3.73
5	15	0.000106	1.44	2.49	6.26
5	20	0.0000096	1.58	3.16	10.1
5	25	0.0000015	1.70	3.80	14.7

TABLE 2. List of species and stocks. Area abbreviations: NAFO, Northwest Atlantic Fisheries Organization: ICES, International Council for the Exploration of the Seas; ICSEAF, International Council for Southeast Atlantic Fisheries.

Species	Area	Stock region or name
Brevoortia tyrannus	NAFO	U.S. Atlantic
Clupea harengus	NAFO	4T(fall), 4WX, AB, CD, EF, GH, I, 4R (spring), 4R (fall), 4T (spring)
	ICES	North Sea, Norway (spring), Iceland (summer) Northern Irish Sea, VIa (south) and VIIbc, VIa (north), Gulf of Finland, Baltic areas (28 and 29S), Gulf of Riga, Southern Central Baltic, Baltic areas 22 and 24, Iceland (spring), Bothnian Bay, Archipelago and Bothnian seas
Engraulis capensis	ICSEAF	1.3 and 1.4, 1.5
Etremeus teres	ICSEAF	1.6
Gadus morhua	NAFO	3 PnRS, 2J3KL, 4X, 4VsW, 3M, 3NO, 3Ps, 4TVn
	ICES	VIIa, Skagerrak, Kattegat, Iceland, NE Arctic, North Sea, Faroe Plateau, Baltic areas 25–32, Baltic areas 22 and 24, VIIde, VIa, Celtic Sea
Hippoglossoides platessoides	NAFO	3LNO, 3Ps
Limanda ferruginae	NAFO	3NO
Melanogrammus aeglefinus	NAFO	4TVW, 4X, 5Z
	ICES	North Sea, Iceland, NE Arctic, Faroe Plateau, VIa
Merluccius bilinearis	NAFO	4VWX, 5Ze, 5Zw6
Merluccius capensis	ICSEAF	1.3 and 1.4, 1.5, 1.6
Micromesistius pouttassou	ICES	Northern area
Pleuronectes platessa	ICES	North Sea, Kattegat, Irish Sea (VIIa)
Pollachius virens	NAFO	4VWX5
	ICES	Iceland, VIa, North Sea, Faroe, NE Arctic
Reinhardtius hippoglossoides	ICES	V and XIV
Sardinops ocellata	ICSEAF	1.6
Scomber japonicus	California	
Scomber scombrus	NAFO	Areas 2–6
	ICES	Western
Solea vulgaris	ICES	North Sea, Celtic Sea, Irish Sea
Trachurus trachurus	ICSEAF	1.3 and 1.4, 1.6
Trisopterus esmarkii	ICES	VIa, North Sea
Urophycis chuss	NAFO	4T

ered to be the first age-class which was available to the commercial fishery.

For each stock there were two measures of recruitment: (1) log-transformed recruitment (number of recruits), denoted by $\log R_t$, and (2) first-differenced log-transformed recruitment, denoted by $\Delta\log R_t$. The first-differenced time series represents year-to-year fluctuations in recruitment (i.e. the change in recruitment from one year to the next) and is determined as

$$(3)\quad \Delta\log R_{t+1/2} = \log R_t - \log R_{t-1}.$$

The first-differenced time series is analogous to removing major trends or cycles from the original time series of recruitment. For each stock considered in this study, we represent recruitment variability as measured by the standard deviation (S) from mean recruitment such that

$$(4)\quad S = \sqrt{\frac{1}{n}\sum_{t=1}^{n}(x_t-\bar{x})^2}$$

where x is the log-transformed measure of recruitment ($\log R_t$) or the first-differenced log-transformed recruitment ($\Delta\log R_t$) and n is the number of observations. In addition to the overall variability in recruitment, the pattern of variation is a key element needed to study the possible causes of fluctuations in recruitment. Myers et al. (1990) calculated the variance ratio

$$(5)\quad \Theta = \frac{S^2_{\Delta\log R_t}}{2\,S^2_{\log R_t}}$$

as a measure of the degree of serial correlation in each recruitment time series. First-differencing will double the variance of a purely random process (i.e. one with no serial correlation). Thus, Θ will be 1 if there is no serial correlation and will be less than 1 if the first-order autocorrelation is between 0 and 1. The average $S_{\log R_t}$, $S_{\Delta\log R_t}$, and Θ for each species was estimated by weighting the value of these statistics for each stock by the length of the time series for that stock and summing over all stocks of that species.

The early life history characteristics specific of each species were obtained from taxonomic or stock summaries (Blaxter and Hempel 1963; Hart 1973; Russell 1976; Fritzsche 1978; Hardy 1978a, 1978b; Johnson 1978; Jones et al. 1978; Martin and Drewry 1978; Fahay 1983; Scott and Scott 1988; Matarese et al. 1989). Whenever possible, we used data for the stock or region for which recruitment information was available; otherwise, we used species averages. The preferred data were the mean lengths of the different stages, but if only a size range was available the midpoint was used. Although weight may be a better measure of organism size (e.g. Peters 1983), length is the most

commonly available measure of size in early life history studies. Species-specific data included egg diameter (Φ), length at hatch (L_0), length at the end of the larval stage (i.e. metamorphosis) (L_m), as well as the change in length during the larval stage ($\Delta L = L_m - L_0$), all measured in millimetres.

Length at the end of the larval stage was chosen to represent the point in the life cycle during which major changes in morphology, behaviour, or habitat occurred. In flatfish, length at which transformation occurred (i.e. rotation of the eye) was the appropriate measure. For gadoids, the average length at which animals begin to leave the water column to enter the demersal phase was taken to indicate the end of the larval phase because metamorphosis per se occurs very slowly and over a wide range of sizes (e.g. Bolz and Lough 1988). Finally, for species that remain pelagic throughout their life cycle, the end of the larval phase was taken as the point at which the larva takes on adult characteristics.

A final aspect of the life history that may influence recruitment variability is the relative duration of the different early life history stages. This can be particularly important if different factors are determining survival during different developmental stages. There is clear evidence that the duration of both egg and larval stages is strongly influenced by size. Pauly and Pullin (1988). Duarte and Alcaraz (1989), and Pepin (1991) found evidence that incubation time increased significantly with increasing egg size. Therefore, species with large eggs will have longer development times than species with smaller eggs, at a given temperature. Furthermore, data from Chambers et al. (1988), Houde (1989), and Pepin (1991) clearly showed that the duration of the larval stage increases as the difference in size between hatch and metamorphosis increases. Consequently, species for which ΔL is large, relative to other species, will have a larval stage of greater duration. If egg size and change in size during the larval stage can be taken as indicators of respective stage durations, under similar environmental constraints, then the relative duration of the two stages (η) can be estimated as

$$(6) \quad \eta = \frac{\Delta L}{\Phi}.$$

The species-specific early life history characteristics are listed in Table 3.

The analysis of the relationship between early life history characteristics and recruitment variability was divided into two stages. First, we used univariate correlation analysis to establish the dominant assocations between life history and recruitment variables. Both Kendall rank (τ) and Pearson product-moment (r) measures of correlation were used to study the assocation between variables. The nonparametric measures of correlation (τ) assumes a monotonic relation — a weaker assumption than linearity. A comparison of nonparametric (τ) and parametric (r) correlation coefficients provides an assessment of the potential influence which outliers might have on the strength of a parametric measure of association. If both measures of correlation indicated a significant relationship, the possible influence of outliers was assumed to be negligible. This was followed by an analysis of residuals from a regression between the life history feature and the measure of recruitment variability which exhibited the strongest association. If a significant residual correlation was apparent, multiple regression was used to investigate the influence of combined stage characteristics. Both univariate and multivariate parametric analyses were performed by weighting the influence of each species by the number of stocks of that species used in estimating the average $S_{\log R_t}$, $S_{\Delta \log R_t}$, and Θ for that species.

TABLE 3. Summary of average early life characteristics for the 21 species used in this study. Symbols: Φ, egg diameter; L_0, length at hatch; L_m, length at metamorphosis; ΔL, change in length during the larval phase; η, change in length during the larval phase relative to the egg diameter ($\Delta L/\Phi$).

Species	Φ (mm)	L_0 (mm)	L_m (mm)	ΔL (mm)	η
Brevoortia tyrannus	1.61	3.0	30	27.0	16.8
Clupea harengus	1.26	7.0	30	23.0	18.0
Engraulis capensis	1.05	3.5	25	21.5	20.5
Etremeus teres	1.35	5.0	34	29.0	21.5
Gadus morhua	1.40	3.0	24	21.0	14.7
Hippoglossoides platessoides	2.15	5.0	25	20.0	9.3
Limanda ferruginae	0.90	2.8	14	11.2	12.4
Melanogrammus aeglefinus	1.43	4.0	28	24.0	16.4
Merluccius bilinearis	0.91	3.0	20	17.0	18.7
Merluccius capensis	1.13	3.3	23	19.7	17.4
Micromesistius pouttassou	1.19	3.2	24	20.8	17.5
Pleuronectes platessa	1.92	2.7	14	11.3	5.9
Pollachius virens	1.12	4.0	25	21.0	18.8
Reinhardtius hippoglossoides	3.40	6.5	24	17.5	5.1
Sardinops ocellata	1.55	4.0	45	41.0	26.5
Scomber japonicus	1.05	3.2	30	26.8	25.5
Scomber scombrus	1.20	3.5	30	26.5	22.1
Solea vulgaris	1.15	3.1	10	6.90	6.0
Trachurus trachurus	0.95	2.4	22	19.6	20.6
Trisopterus esmarkii	1.12	3.3	24	20.7	18.5
Urophycis chuss	0.75	3.0	24	21.0	28.0

Results

Recruitment and Survival

Comparison of the variability in survival from egg to age 1+ with the variability in recruitment from Garrod's (1982) summary of the number of eggs spawned and the number of recruits shows a significant correlation ($S_{\log R_t} = 0.04 + 0.90 S_{\log R_t/E_t}$, $r = 0.81$, $p < 0.01$, $n = 12$, where $S_{\log R_t}$ and $S_{\log R_t/E_t}$) are the standard deviation of recruitment and survival, respectively) (Fig. 1). This result clearly indicates that variations in recruitment, relative to their long-term mean, can be used as a measure of variations in survival during the prerecruit phase.

Recruitment Variability and Size

There is no evidence that egg diameter (Φ) (Table 4; Fig. 2) or the length at hatch (L_0) (Table 4; Fig. 3) was the principle factor determining differences in recruitment variability. This indicates that neither the initial energy investment by the parents nor the initial foraging ability of the larvae, which are related to egg and larval fish size, respectively (Miller et al. 1988; Duarte and Alcaraz 1989), is the principal factor determining differences in recruitment variability between species.

The change in length from hatch to metamorphosis ($\Delta L = L_m - L_0$) shows a highly significant correlation with recruitment variability ($r = 0.59$, $p < 0.01$, $\tau = 0.39$, $p < 0.05$, $n = 21$) (Table 4; Fig. 4) which is greater than the influence of the length at metamorphosis (Table 4). There is also a strong correlation between the standard deviation in

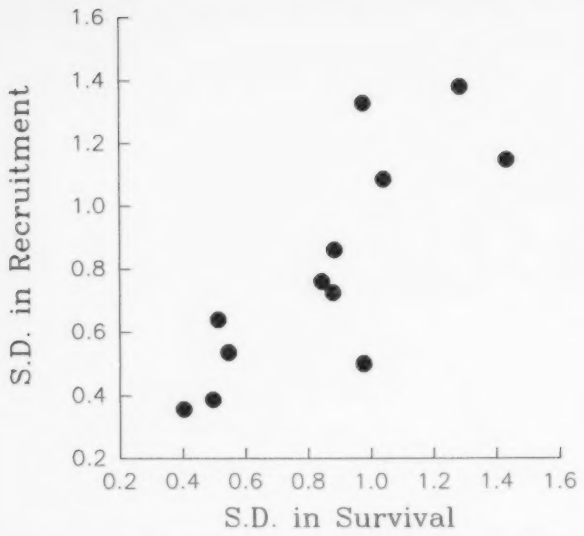

FIG. 1. Scatter plot showing the relationship between the standard deviation in survival ($S_{\log (R_t/E_t)}$) and the standard deviation in recruitment ($S_{\log R_t}$).

recruitment and the relative size/durationof larval and egg stages (η) (Table 4). The general implication is that the longer the duration of the larval phase, in either size or time, the greater the number of factors (e.g. trophic levels) that may influence survival and hence recruitment variability. The regression describing the standard deviation in log-transformed recruitment ($S_{\log R_t}$) in relation to change in length during the larval phase (ΔL) is

$$(7) \quad S_{\log R_t} = -0.11 + 0.022\Delta L$$

($r = 0.59, p < 0.01, n = 21$). Residuals from this regression are not significantly correlated with either egg diameter ($r = -0.30, p > 0.1, \tau = -0.15, p > 0.2$), length at hatch ($r = 0.10, p > 0.5, \tau = -0.04, p > 0.5$), or length at metamorphosis ($r = 0.03, p > 0.5, \tau = -0.07, p > 0.5$).

The change in length during the larval stage is determined by the initial size and the length at metamorphosis. To determine how these two components contribute to recruitment variability, we estimated the multiple regression parameters that yield

$$(8) \quad S_{\log R} = -0.12 - 0.011L_0 + 0.021L_m$$

($r = 0.60, p < 0.05, n = 21$). Only the regression coefficient for length at metamorphosis (L_m) was significantly different from 0 ($t = 2.68, p < 0.05$). The intercept and the regression coefficient for the length at hatch (L_0) were not significantly different from 0 ($p = 0.46$ and $p = 0.65$, respectively).

The standard deviation of the first-differenced log-transformed recruitment data ($S_{\Delta \log R}$) shows a pattern of correlations that is consistent with the results for the log-transformed recruitment data. Neither egg diameter nor length at hatch was significant correlated with the standard deviation of the differenced data (Table 4). The standard deviation of the changes in length during the larval period shows a weaker correlation with the differenced data than was observed in the undifferenced data.

The variance ratio (Θ), which decreases as the degrees of serial correlation increases, is significantly correlated with egg diameter ($r = 0.46, p < 0.05, \tau = 0.34, p < 0.05, n = 21$) (Table 4) and appears not to be strongly correlated with the change in length during the larval phase ($r = -0.31, p > 0.1, \tau = -0.08, p > 0.1, n = 21$). However, there is a highly significant relationship ($r = -0.54, p < 0.01, \tau = -0.34, p < 0.05, n = 21$) between the variance ratio (Θ) and the duration of the larval stage relative to that of the egg stage (η) (Fig. 5). Species that produce large eggs relative to the change in size during the larval phase have more random recruitment pattern than species that produce relatively smaller eggs.

Discussion

The purpose of this paper has been to determine whether there is a correspondence between recruitment variability and early life history characteristics. The simplest hypothesis, based on life history theory, has been that species which produce many small eggs will have greater variations in survival than species which produce few large eggs (Smith and Fretwell 1974; Stearns 1980, 1983). The results of this study do not support this hypothesis; both egg size and initial size of offspring have little influence on recruitment variations. It has been assumed by many that small offspring are more susceptible to variations in food abundance (Blaxter and Hempel 1963; Miller et al. 1988) and are more vulnerable to predators than larger ones (Pepin et al. 1987; Miller et al. 1988). Many studies have proposed a link between environmental conditions (mainly food availability) at the time of first feeding and survival (Hjort 1914; Cushing 1975; Lasker 1978). However, only a few have been able to demonstrate such correspondence (e.g. Peterman and Bradford 1987). It appears that during the egg and early larval phase, the initial size of different species does not have a sufficiently

TABLE 4. Pearson product-moment correlation coefficient (r) and Kendall's nonparametric correlation coefficient (τ) for the relationship between the various early life history characteristics and average features of recruitment variability. Significance levels: $*p < 0.05$; $**p < 0.01$; ns, not significant.

	Pearson product-moment correlation coefficient (r)			Kendall's nonparametric correlation coefficient (τ)		
Characteristic	$S_{\log R}$	$S_{\Delta \log R}$	Θ	$S_{\log R}$	$S_{\Delta \log R}$	Θ
Egg diameter	−0.27 ns	−0.20 ns	0.46*	−0.08 ns	0.04 ns	0.34*
Length at hatch	0.27 ns	0.30 ns	0.08 ns	0.14 ns	0.22 ns	0.11 ns
Length at metamorphosis	0.56**	0.46*	−0.24 ns	0.32*	0.32*	−0.06 ns
ΔL	0.59**	0.43*	−0.31 ns	0.39*	0.36*	−0.08 ns
$\eta = \Delta L/\Phi$	0.60**	0.44*	−0.56**	0.47**	0.32*	−0.34*

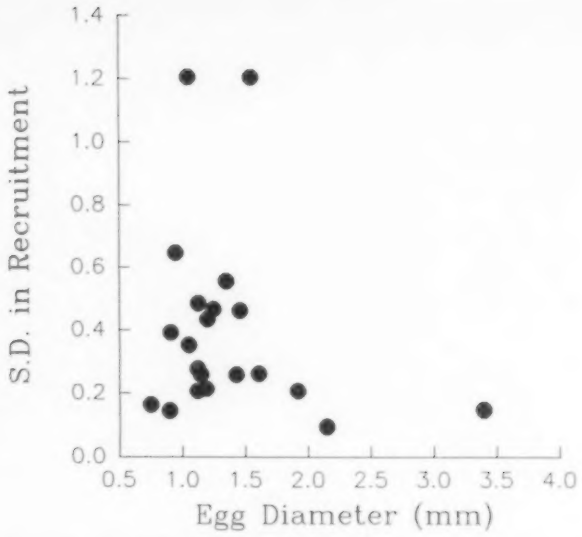

FIG. 2. Scatter plot of the standard deviation in recruitment ($S_{\log R_t}$) in relation to egg diameter (Φ).

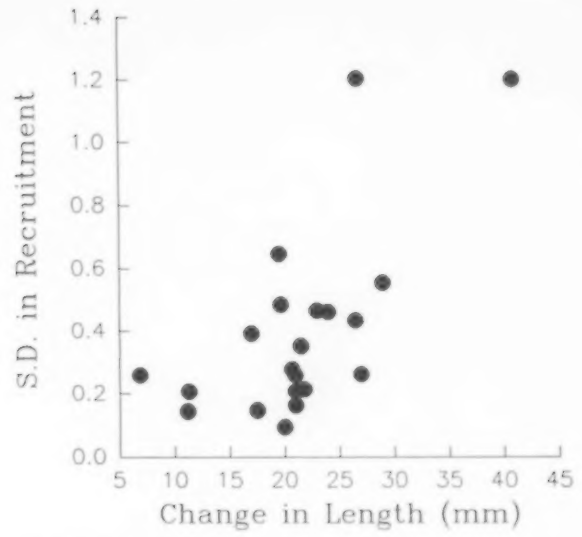

FIG. 4. Scatter plot of the standard deviation in recruitment ($S_{\log R_t}$) in relation to the change in length during the larval phase (ΔL).

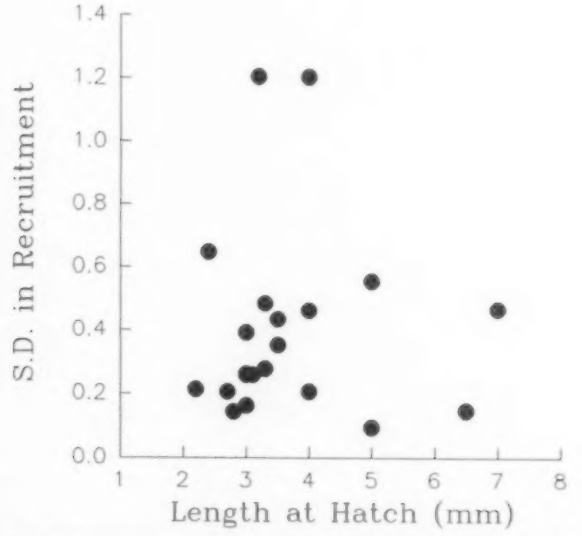

FIG. 3. Scatter plot of the standard deviation in recruitment ($S_{\log R_t}$) in relation to length at hatch (L_0).

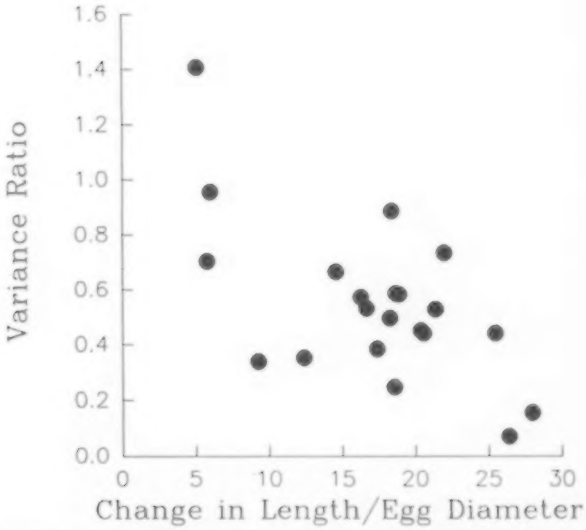

FIG. 5. Scatter plot of the variance ratio of recruitment (Θ) in relation to η, the change in length during the larval phase (ΔL), relative to the diameter of the egg (Φ).

strong influence on their vulnerability to fluctuations in environmental conditions to be apparent in the overall survival, which is consistent with Pepin's (1991) conclusions. Pepin (1991) found no significant relationship between the cumulative stage-specific mortality and the size of fish eggs and yolk-sac larvae. It is possible that the average size of hatchlings is such that environmental conditions are generally suitable to insure adequate survival during the egg and early larval stages and that catastrophic or beneficial events, which may lead to year-class failure or success, are infrequent or that their influence is independent of size.

The evidence presented shows that overall recruitment variability is significantly correlated with the change in length during the larval period, which provides a measure of the duration of this stage of the life cycle. As the duration of the larval stage increases, cumulative mortality increases (Smith 1985; Houde 1987; Beyer 1989; Pepin 1991) which results in greater recruitment variability. This is consistent with the suggestion that year-class strength is determined during the phase of the life cycle during which the greatest variability in cumulative mortality occurs. As larvae increase in size, their prey and predator fields change. As the number of prey and/or predator fields increases, the potential for variations in development and mortality rates increases, thus potentially increasing variations in cumulative mortality.

Multiple regression analysis shows that recruitment variability significantly increases with increasing length at metamorphosis but that there is no significant relationship with length at hatch. Chambers et al. (1988) found that the coefficient of variation in the length at hatch of winter flounder (*Pseudopleuronectes americanus*), reared in the laboratory, was significantly less than the coefficient of variation in the length at metamorphosis. They also found a similar pattern in the ages at hatch and at metamorphosis and that the age at which these transitions occurred was significantly more variable that length at transition. Consequently, length (L_m) and age at metamorphosis which have the greatest relative variability within a population will have the greatest influence in determining variations in larval fish survival, as was found in this study. Because length at hatch and length at metamorphosis are poorly correlated (Chambers et al. 1988; the correlation between L_0 and L_m for the 21 species used in this study is $r = 0.36$, $0.1 > p > 0.05$), the effects of variation in size at metamorphosis on survival may obscure the effect of variations in length during the early larval stage. Alternatively, failure to find a significant effect of initial size on recruitment variability in this study may be due to errors in estimating length at hatch. Subtle factors leading to measurement errors (e.g. shrinkage and body distortion due to capture and preservation) have been shown to have a greater impact on estimated length of small larvae relative to larger ones (Hay 1982; Fowler and Smith 1983; Glenn and Mathias 1987). Consequently, inaccuracies in estimated initial size may obscure the significance of this variable on recruitment variability. Studies of early life history stages, particularly larval fish, should attempt to estimate the mean and variance in size of the young larvae with as much accuracy as possible.

The serial correlation in recruitment from year to year is linked to early life history characteristics. Most recruitment time series show some degree of serial correlation (Koslow 1984; Shepherd et al. 1984; Myers et al. 1990). We outlined earlier that increasing the size of the egg stage relative to the change in length during the larval stage may be taken as an index of the relative duration of those two stages. Our results indicate that increasing the duration/size (Φ) of the egg stage relative to the duration/size of the larval stage (ΔL) leads to a greater degree of randomness in recruitment (i.e. less serial correlation) (Table 4). This association may result from the differential effect of a given factor on the survival of egg and larval stages. For example, Pepin (1991) found that increasing temperature led to decreasing survival during the egg stage, increasing survival during the stage of yolk absorption, and no significant effect on the survival of feeding larvae. Furthermore, different predators may feed differentially on eggs and larvae (see Bailey and Houde 1989 for a review). If such patterns occur with respect to the effect of other environmental factors, changing the distribution of the time spent in each early life history stage may allow different species to show different levels of overall vulnerability to environmental fluctuations, as reflected by the pattern and variability in recruitment. Species that have life history cycles with prolonged larval phases relative to the duration of other early life history stages may therefore be more vulnerable to any given environmental factor(s) than species with short larval phases. Because most of the variability in marine environments is contained in long-term (decadal scales and longer) cycles (Horne and Platt 1984), species with a prolonged larval stage, relative to the duration of the egg stage, may show a greater degree of serial correlation in recruitment that would reflect long-term changes in environmental factors. Caution must be used in interpreting the relationship shown in Fig. 5. It is possible that the degree of serial correlation may be increased if there are aging errors in the data used to calculate recruitment using sequential population analysis (Myers and Drinkwater 1989). However, our review of the data suggests that this may not be a major limitation of the information presented.

Other studies have also shown correspondence between temporal variability in population abundance and life's history characteristics. In a variety of insect groups, temporal variability in abundance is positively correlated with fecundity (Spitzer and Leps 1988) and geographical range (Gaston and Lawton 1988), while it has been found to be negatively correlated with longevity (Connell and Sousa 1983), size (Gaston and Lawton 1988), and polyphagy (Redfern and Pimm 1988). No difference in the variability in abundance between the tropical and temperate insect populations has been found (Wolda 1978, 1983). Fairweather (1988) demonstrated that predation increased the temporal variability of abundance of intertidal invertebrates. Myers (1991) showed that variability in recruitment of marine fish is greater in populations at the northern and southern limits of the species' range. Future studies should attempt to assess the relative importance of variations in several life history characteristics in determining variations in population abundance or recruitment.

It has been suggested that estimating the number of fish larvae is a poor indicator of year-class strength (Sissenwine 1984; Peterman et al. 1988). This has led to proposals that egg and larval sampling programs would provide only a partial understanding of the sources of variations in recruitment (Peterman et al. 1988; Walters and Collie 1988). It is possible that limitations in gear efficiency or avoidance of nets by large larvae may limit estimates of abundance to a relatively short period of the early life history. Although our analysis is correlative, and thus is designed to provide hypotheses for future research, the evidence suggests that variations in the change in size between hatch and metamorphosis may contribute significantly to recruitment variability. Our hypotheses could be further tested by examining data for other species, or by testing the hypotheses among stocks of the same species. The evidence presented in this study suggests that the entire early life history (i.e. both egg and larval stages) is an important determinant of recruitment variability to populations of commercial marine fish species. Thus, to study the significance of any given factor(s) on recruitment variability, it is necessary to design research objectives that encompass the entire early life rather than concentrate principally on the early developmental stages.

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References

BAGENAL, T. B. 1971. The interrelation of the size of fish eggs, the date of spawning and the production cycle. J. Fish Biol. 3: 207–219.

BAILEY, K. M., AND E. D. HOUDE. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. 26: 1–83.

BEYER, J. 1989. Recruitment stability and survival: simiple size-specific theory with examples from the early life dynamics of marine fish. Dana 7: 45–147.

BLAXTER, J. H. S., AND G. HEMPEL. 1963. The influence of egg size on herring larvae (*Clupea harengus*). J. Cons. Int. Explor. Mer 28: 211–240.

BOLZ, G. R., AND R. G. LOUGH. 1988. Growth through the first six months of Atlantic cod, *Gadus morhua*, and haddock, *Melanogrammus aeglefinus*, based on daily otolith increments. Fish. Bull. 86: 223–235.

BUTTERWORTH, D. S. 1983. Assessment and management of pelagic stocks in the southern Benguela region, p. 329–406. *In* G. D. Sharp and J. Csirke [ed.] Proceedings of the expert consultation to examine changes in abundance and species of neritic fish resources, San José, Costa Rica, 18–29 April 1983, FAO Fish. Rep. 291(2).

CHAMBERS, R. C., W. C. LEGGETT, AND J. A. BROWN. 1988. Variation in and among early life history traits of laboratory-reared winter flounder *Pseudopleuronectes americanus*. Mar. Ecol. Prog. Ser. 47: 1–15.

COLE, L. C. 1954. The population consequences of life history phenomena. Q. Rev. Biol. 29: 103–137.

CONNELL, J. F., AND W. P. SOUSA. 1983. On the evidence needed to judge ecological stability or persistence. Am. Nat. 121: 789–824.

CRAWFORD, J. M., P. A. SHELTON, AND L. HUTCHINGS. 1983. Aspects of variability of some neritic stocks in the southern Benguela system, p. 407–448. *In* G. D. Sharp and J. Csirke [ed.] Proceedings of the expert consultation to examine changes in abundance and species of neritic fish resources, San José, Costa Rica, 18–29 April 1983. FAO Fish. Rep. 291(2).

CUSHING, D. H. 1975. Marine ecology and fisheries. Cambridge University Press, Cambridge, London, New York, and Melbourne. 278 p.

DUARTE, C. M., AND M. ALCARAZ. 1989. To produce many small or few large eggs: a size-independent reproductive tactic of fish. Oecologia 80: 401–404.

FAHAY, M. P. 1983. Guide to the early stages of marine fishes occurring in the western north Atlantic, Cape Hatteras to the southern Scotian Shelf. J. Northwest Atl. Fish. Sci. 4: 423 p.

FAIRWEATHER, P. G. 1988. Predation can increase variability in the abundance of prey on seashores. Oikos 53: 87–92.

FOWLER, G. M., AND S. J. SMITH. 1983. Length changes in silver hake (*Merluccius bilinearis*) larvae: effects of formalin, ethanol, and freezing. Can. J. Fish. Aquat. Sci. 40: 866–870.

FRITZSCHE, R. A. 1978. Development of fishes of the mid-Atlantic Bight: an atlas of egg, larval and juvenile stages. Vol. 5. *Chaetodontidae* through *Ophidiidae*. Fish and Wildlife Service, Washington, DC. 340 p.

GARROD, D. J. 1982. Stock and recruitment — again. Minist. Agric. Fish. Food Dir. Fish. Res. Fish. Res. Tech. Rep. No. 68: 22 p.

GARROD, D. J., AND J. M. COLEBROOK. 1978. Biological effects of variability in the North Atlantic Ocean. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 173: 128–144.

GASTON, K. J., AND J. H. LAWTON. 1988. Patterns in the distribution and abundance of insect populations. Nature (Lond.) 331: 709–712.

GLENN, C. L., AND J. A. MATHIAS. 1987. Body shrinkage in young walleye, *Stizostedion vitreum*), preserved with AFA, formalin, ethanol and quick freezing. Can. Field-Nat. 101: 408–414.

HARDY, J. D. 1978a. Development of fishes of the mid-Atlantic Bight: an atlas of egg, larval and juvenile stages. Vol. 2. *Anguillidae* through *Syngnathidae*. Fish and Wildlife Service, Washington, DC. 458 p.

1978b. Development of fishes of the mid-Atlantic Bight: an atlas of egg, larval and juvenile stages. Vol. 3. *Aphredoderidae* through *Rachycentridae*. Fish and Wildlife Service, Washington, DC. 394 p.

HART, J. L. 1973. Pacific fishes of Canada. Bull. Fish. Res. Board Can. 180: 740 p.

HAY, D. E. 1982. Fixation shrinkage of herring larvae: effects of salinity, formalin concentration, and other factors. Can. J. Fish. Aquat. Sci. 39: 1138–1143.

HJORT, J. 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 20: 1–228.

HORNE, E. P. W., AND T. PLATT. 1984. The dominant space and time scales of variability in the physical and biological fields on continental shelves. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 183: 8–19.

HOUDE, E. D. 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. 2: 17–29.

1989. Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish. Bull. 87: 471–496.

JOHNSON, G. D. 1978. Development of fishes of the mid-Atlantic Bight: an atlas of egg, larval and juvenile stages. Vol. 4. *Carangidae* through *Ephippidae*. Fish and Wildlife Service, Washington, DC. 314 p.

JONES, P. W., F. D. MARTIN, AND J. D. HARDY. 1978. Development of fishes of the mid-Atlantic Bight: an atlas of egg, larval and juvenile stages. Vol. 1. *Acipenseridae* through *Ictaluridae*. Fish and Wildlife Service, Washington, DC. 366 p.

JONES, R. 1983. The decline of herring and mackerel and the associated increase in other species in the North Sea, p. 507–520. *In* G. D. Sharp and J. Csirke [ed.] Proceedings of the expert consultation to examine changes in abundance and species of neritic fish resources, San José, Costa Rica, 18–29 April 1983. FAO Fish. Rep. 291(2).

KOSLOW, J. A. 1984. Recruitment patterns in Northwest Atlantic fish stocks. Can. J. Fish. Aquat. Sci. 41: 1722–1729.

KOSLOW, J. A., K. R. THOMPSON, AND W. SILVERT. 1987. Recruitment to Northwest Atlantic cod and haddock stocks: influence of stock size and climate. Can. J. Fish. Aquat. Sci. 44: 26–39.

LASKER, R. 1978. The relation between oceanographic conditions and larval anchovy food in the California Current: identification of factors contributing to recruitment failure. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 178: 375–388.

LEGGETT, W. C., K. T. FRANK, AND J. E. CARSCADDEN. 1984. Meteorological and hydrographic regulation of year-class strength in capelin (*Mallotus vilosus*). Can. J. Fish. Aquat. Sci. 41: 1193–1201.

MACCALL, A. D. 1983. Variability of pelagic stocks off California, p. 101–112. *In* G. D. Sharp and J. Csirke [ed.] Proceedings of the expert consultation to examine changes in abundance and species of neritic fish resources, San José, Costa Rica, 18–29 April 1983. FAO Fish. Rep. 291(2).

MARKLE, D. F., AND L.-A. FROST. 1985. Comparative morphology, seasonality, and a key to planktonic fish eggs from the Nova Scotian shelf. Can. J. Zool. 63: 246–257.

MARTIN, F. D., AND G. E. DREWRY. 1978. Development of fishes of the mid-Atlantic Bight: an atlas of egg, larval and juvenile stages. Vol. 6 *Stromateidae* through *Ogcocephalidae*. Fish and Wildlife Service, Washington, DC. 416 p.

MATARESE, A. C., A. W. KENDALL, D. M. BLOOD, AND B. M. VINTER. 1989. Laboratory guide to early life history stages of Northeast Pacific fishes. NOAA Tech. Rep. NMFS 80: 652 p.

MAY, R. C. 1974. Larval immortality in marine fishes and the critical period concept, p. 3–20. *In* J. H. S. Blaxter [ed.] The early life history of fish. Springer-Verlag, Berlin.

MCGURK, M. D. 1986. Natural mortality of marine pelagic fish eggs and larvae: role of spatial patchiness. Mar. Ecol. Prog. Ser. 34: 227–242.

MILLER, T. J., L. B. CROWDER, J A. RICE, AND E. A. MARSCHALL. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. 45: 1657–1670.

MYERS, R. A. 1991. Population variability of juvenile fish and the range of cod, haddock and herring in the North Atlantic. J. Anim. Ecol. (In press)

MYERS, R. A., W. BLANCHARD, AND K. R. THOMPSON. 1990. Summary of north Atlantic fish recruitment 1942–1987. Can. Tech. Rep. Fish. Aquat. Sci. 1743: 108 p.

MYERS, R. A., AND K. DRINKWATER. 1989. The influence of Gulf Stream warm core rings on recruitment of fish in the Northwest Atlantic. J. Mar. Res. 47: 635–656.

PAULY, D., AND R. S. V. PULLIN. 1988. Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. Environ. Biol. Fishes 22: 261–271.

PEPIN, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Can. J. Fish. Aquat. Sci. 48: 503–518.

PEPIN, P., S. PEARRE, AND J. A. KOSLOW. 1987. Predation on larval fish by Atlantic mackerel, *Scomber scombrus*, with a comparison of predation by zooplankton. Can. J. Fish. Aquat. Sci. 44: 2012–2018.

PETERMAN, R. M., AND M. J. BRADFORD. 1987. Wind speed and mortality rate of a marine fish, the northern anchovy (*Engraulis mordax*). Science (Wash., DC) 235: 354–356.

PETERMAN, R. M., M. J. BRADFORD, N. C. H. LO, AND R. D. METHOT. 1988. Contributions of early life stages to interannual variability in recruitment of northern anchovy (*Engraulis mordax*). Can. J. Fish. Aquat. Sci. 45: 8–16.

PETERS, R. H. 1983. The ecological significance of body size. Cambridge University Press, Cambridge. 329 p.

PETERSON, I., AND J. S. WROBLEWSKI. 1984. Mortality rates of fishes in the pelagic ecosystem. Can. J. Fish. Aquat. Sci. 41: 1117–1120.

REDFERN, A., AND S. L. PIMM. 1988. Population variability and polyphagy in herbivorous insect communities. Ecol. Monogr. 58: 39–55.

ROTHSCHILD, B. J., AND G. T. DINARDO. 1987. Comparison of recruitment variability and life history data among marine and anadromous fishes. Am. Fish. Soc. Symp. 1: 531–546.

RUSSELL, F. S. 1976. The eggs and planktonic stages of British marine fishes. Academic Press, London, UK. 524 p.

SCOTT, W. B., AND M. G. SCOTT. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. 219: 731 p.

SHEPHERD, J. G., J. G. POPE, AND R. D. COUSENS. 1984. Variations in fish stocks and hypotheses concerning their links with climate. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 185: 255–267.

SISSENWINE, M. P. 1984. Why do fish populations vary?, p. 59–94. *In* R. M. May [ed.] Exploitation of marine communities. Report of the Dalhem workshop on exploitation of marine communities, Berlin 1984. Springer Verlag, Berlin.

SMITH, C. C., AND S. D. FRETWELL. 1974. The optimal balance between size and number of offspring. Am. Nat. 108: 199–206.

SMITH, P. E. 1985. Year-class strength and survival of O-group clupeoids. Can. J. Fish. Aquat. Sci. 42(Suppl. 1): 69–82.

SPITZER, K., AND J. LEPS. 1988. Determinants of temporal variation in moth abundance. Oikos 53: 31–36.

STEARNS, S. C. 1980. A new view of life-history evolution. Oikos 35: 266–281.

1983. The influence of size and phylogeny on patterns of covariation among life-history traits in mammals. Oikos 41: 173–187.

SUTCLIFE, W. H., R. H. LOUCKS, K. F. DRINKWATER, AND A. R. COOTE. 1983. Nutrient flux onto the Labrador Shelf from Hudson Strait and its biological consequences. Can. J. Fish. Aquat. Sci. 40: 1692–1701.

WALTERS, C. J., AND J. S. COLLIE. 1988. Is research on environmental factors useful to fisheries management? Can. J. Fish. Aquat. Sci. 45: 1848–1854.

WARE, D. M. 1977. Spawning time and egg size of Atlantic mackerel, *Scomber scombrus*, in relation to the plankton. J. Fish. Res. Board Can. 34: 2308–2315.

WOLDA, H. 1987. Fluctuations in abundance of tropical insects. Am. Nat. 112: 1017–1045.

1983. Long-term stability of tropical insect populations. Res. Popul. Ecol. Suppl. 3: 112–126.

Effects of Inorganic Cations on Na^+ Adsorption to the Gill and Body Surface of Rainbow Trout, *Oncorhynchus mykiss*, in Dilute Solutions

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Handy, R. D., and F. B. Eddy. 1991. Effects of inorganic cations on Na^+ adsorption to the gill and body surface of rainbow trout, *Oncorhynchus mykiss*, in dilute solutions. Can. J. Fish. Aquat. Sci. 48: 1829–1837.

Using the shortest practical exposure time (15 s) to 0.2 mmol·L^{-1} NaCl solutions containing ^{22}Na, over 80% of the Na^+ taken up by rainbow trout, *Oncorhynchus mykiss*, was located on the gill or body surface (adsorption). Influx to the blood (absorption) was less than 20% of the total taken up, even though Na^+ entry into the blood was detected in less than 30 s. Sodium adsorption to the fish surface was strongly reduced by micromolar concentrations of Ca^{2+} or by acid in the range pH 7–5. At higher NaCl concentrations (2 mmol·L^{-1} in the medium, Ca^{2+} effects on adsorption were greatly attenuated. Aluminum had a negligible effect on adsorption at pH 4. It is concluded that adsorption has an important role in presenting Na^+ to the apical surface of gill ion transporting cells, since conditions which inhibit the branchial influx of Na^+ also reduce Na^+ adsorption. The results suggest that adsorption is controlled by ion exchange events at gill surface ligands, and by the effects of other cations on the mobility of Na^+ in the bulk water. Adsorption phenomena are likely to influence ionic regulation during normal fluctuations in environmental conditions.

Chez des truites arc-en-ciel, *Oncorhynchus mykiss*, exposées pendant la période la plus courte possible (15 s) à 0,2 mmol·L^{-1} d'une solution de NaCl contenant du ^{22}Na, on a constaté que plus de 80 % du Na^+ reçu se retrouvait sur les branchies ou sur la surface du corps (adsorption). La quantité retrouvée dans le sang (absorption) représentait moins de 20 % de la quantité totale reçue, même si l'introduction du Na^+ dans la circulation était détectée en moins de 30 s. L'adsorption de Na^+ sur la surface du corps s'est trouvée nettement réduite par du Ca^{2+} à une concentration micromolaire ou lorsque le pH était dans la plage d'acidité de 7 à 5. Aux concentrations de NaCl plus élevées (2 mmol·L^{-1} dans le milieu), les effets du Ca^{2+} sur l'adsorption se sont beaucoup atténués. L'aluminium n'a eu qu'un effet négligeable sur l'adsorption à un pH de 4. On en conclut que l'adsorption joue un rôle important dans l'apport de Na^+ à la surface apicale des cellules transporteuses d'ions des branchies, puisque les conditions entraînant l'inhibition du flux branchial de Na^+ provoquent aussi la baisse de l'adsorption du Na^+. Ces résultats portent à penser que l'adsorption est réglée par des phénomènes d'échanges d'ions dans les ligands de la surface des branchies et par les effets des autres cations sur la mobilité du Na^+ dans la masse d'eau. Les phénomènes d'adsorption influent probablement sur la régulation ionique durant les fluctuations normales des conditions du milieu.

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Fish kills in soft water have led to extensive studies on the physiological nature of acid and metal toxicity (McDonald 1983; Spry and Wood 1985; Booth et al. 1988; Goss and Wood 1988; Reid and McDonald 1988). Damage to the gill epithelium often results in the loss of plasma electrolytes and the inhibition of Na^+ uptake (McDonald 1983; Booth et al. 1988). Metal toxicity is also influenced by water quality. Increasing water hardness by the addition of Ca^{2+} reduces Al toxicity (Brown 1983; Booth et al. 1988), while the toxicities of Cu, Zn, and Cd are ameliorated by the presence of H^+, which possibly interferes with metal uptake (Cusimano et al. 1986).

However, despite the evidence for gross physiological effects of waterborne metal toxicants on ion balance, there is limited information on events at the gill and body surface. Water quality influences on Na^+ availability to the gill microenvironment remain uninvestigated. The gill microenvironment is not fully described, but is probably formed from the extracellular material (extracellular matrix) on the apical membrane and an associated unstirred water layer. Mucus may also be present in stress situations, but is apparently absent from the normal gill microenvironment (Handy and Eddy 1989, 1991). Physicochemical interactions between the gill epithelial cell surface, the metal toxicant, and the bulk water are likely to be of importance in determining the nature of metal toxicity.

The first step in branchial Na^+ influx is the provision of a readily available supply of Na^+ at the apical membrane surface. This will be influenced not only by water quality, but also the number and type of metal-binding ligands on the gill cell apical surface, as well as the chemical speciation of the toxicant and its interactions with cell surface metal-binding ligands (review by Campbell and Stokes 1985). The branchial microenvironment is also different from the bulk water (Playle and Wood 1989), but the extent and mechanisms of bulk water quality influences on Na^+ levels in the branchial microenvironment are unknown.

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In waters containing complex polyelectrolytes, such as hard freshwaters, the availability of an ion to organisms will depend partly on ionic mobility which is influenced by charge, hydrated ionic radius, and also the number and species of other surrounding ions (Robinson and Stokes 1965). In this situation the interactions between two cation species would be difficult to analyse. However, in low ionic strength electrolyte solutions (e.g. 0.2 mmol·L^{-1} NaCl solution or certain soft waters), each ion is considered to be at "infinite" dilution and its mobility is determined chiefly by its charge and hydrated ionic radius rather than by influences of neighbouring ions (Robinson and Stokes 1965), so providing a much simpler system for examining the "competitive" effects of another single ionic species (secondary ion). The method described in this paper was developed using dilute NaCl solutions such that the influence of an additional ionic species, as well as bulk water chemistry, on Na^+ availability to the gill and body surface could be examined. This is particularly applicable to soft waters containing metal toxicants, which are often poorly buffered and of low ionic strengths.

The uptake of Na^+ by the gill will involve two main processes, adsorption of Na^+ onto the gill surface (Pagenkopf 1983) and absorption into the blood via branchial influx (e.g. McDonald and Rogano 1986). The method was further developed, using short exposure periods to NaCl solutions, so that surface effects or adsorption could be examined with minimal interference from Na^+ influx (absorption) to internal tissues. This paper makes a first attempt at quantifying Na^+ adsorption phenomena at the gill surface and indicates some of the environmental factors which influence the capture of Na^+ at the branchial epithelium surface.

Methods

Experimental Animals

Rainbow trout, *Oncorhynchus mykiss*, weighing between 3 and 12 g were obtained from Cloan Hatchery, Glen Devon, Scotland, and maintained in aquaria with aerated flowing freshwater containing 0.2 mmol Na^+, 0.01 mmol K^+, 0.13 mmol Ca^{2+}, 0.006 mmol Mg^{2+}, and 0.2 mmol Cl^-·L^{-1} with pH values and temperature ranging between 7.4 and 8.2 and 7 and 10°C, respectively. Fish were fed daily with commercial trout pellets and exposed to a 12L:12D photoperiod.

Experimental Protocol

The process of Na^+ adsorption onto the fish surface from NaCl solutions was examined using medium radiolabelled with ^{22}Na. Fish were removed from aquaria and then rinsed in unlabelled NaCl solutions before and after exposure to the radiolabelled medium. Thus, fish were exposed sequentially to three solutions: (1) 30 L of 0.2 mmol·L^{-1} NaCl for 5 s, (2) 500 mL of 0.2 mmol·L^{-1} NaCl containing 10 μCi (370 kBq) of ^{22}Na (Amersham), and (3) 30 L of 0.2 mmol·L^{-1} NaCl for 5 s.

Exposure to the first solution ensured that ions other than Na^+ and Cl^- present in residual freshwater associated with the fish surface were not transferred into the radiolabelled solution. The final rinse (solution 3) in a separate unlabelled solution ensured removal of unbound radiolabel from the body surface and branchial chamber. The rinse time of 5 s allowed approximately seven breaths, thus ensuring replacement of any residual medium in the branchial chamber. Since all solutions contained 0.2 mmol·L^{-1} NaCl, fish were exposed to consistent Na^+ concentrations throughout each experiment.

All experimental solutions were initially adjusted to pH 7 with a few drops of 0.1 mmol·L^{-1} Trizma Base (Sigma). In all experiments, fish were exposed individually to the solutions and were exposed once only to each solution and then killed with a blow to the head, placed in preweighed tubes, and weighed before gamma counting (LKB-WALLAC 80 000 automatic gamma counter, LKB Produkter, Sweden). All pH measurements were made using a low-conductivity combination pH electrode (Russell pH, Auchtermuchty, Scotland) connected to an acid–base analyser (Radiometer, Copenhagen), while Na^+ and Ca^{2+} concentrations (see below) were determined by atomic absorption spectrophotometry (Pye Unicam SP9).

Several types of experiment were performed based on the protocol described above. Experiments were conducted with immobilised fish to demonstrate Na^+ adsorption, using the body surface alone to exclude interference from branchial absorption. (2) Experiments were conducted with free-swimming fish to establish optimum surface adsorption and minimal internal absorption and to determine the effects of changes in H^+, Ca^{2+}, and Al concentration on Na^+ adsorption to the gill and body surface.

Experiments with Immobilised Fish

These experiments demonstrate Na^+ adsorption by the body surface alone. Active influx can be neglected, since the gills were not exposed to any solutions and the skin has a negligible capacity for active ion absorption (Krogh 1937). Fish were immobilised by a blow to the head and then held with fine forceps posterior to the operculum. The bodies were then dipped sequentially (without immersing the gills) in the NaCl solutions described, spending 15 s in the radiolabelled medium.

Two experiments were performed, one examining H^+ and the other Ca^{2+} effects on adsorption by the body surface. Either H^+ or Ca^{2+} was added to the radiolabelled medium. In the H^+ experiment, groups of seven fish were individually immersed in the radiolabelled medium at a pH of 7, 6, 5, 4, or 3. In the Ca^+ experiment, additions of Ca^{2+} gave concentrations in the range 0–2.5 mmol·L^{-1} (see Table 1 for details of solutions). Water samples (2 mL) were collected after each acid or Ca^{2+} addition for gamma counting as well as determination of Na^+ and Ca^+ concentrations.

Experiments with Free-Swimming Fish

Minimizing Na^+ absorption to internal tissues

In experiments with free-swimming fish the gills as well as the body surface were exposed to the radiolabelled solution; thus, the possibility of branchial Na^+ influx existed. Experiments were performed to establish the time course of Na^+ influx, if any, into the blood and livers within a period of 30 s and to determine the optimum exposure period to the radiolabelled solution so that surface effects predominate. Handling stress influences on Na^+ influx and adsorption are minimal in such short-term experiments, since stress-related elevations of plasma catecholamines and cortisol occur over 30 min and several hours, respectively (Mazeaud and Mazeaud 1981; Pickering et al. 1982), rather than seconds.

Trout were individually netted from the aquarium water and immersed sequentially in the three NaCl solutions described (pH 7, zero Ca^{2+}) and kept in the radiolabelled solution for 0,

TABLE 1. Composition of media, species, and form of secondary ion additions to which rainbow trout were exposed for Na^+ adsorption experiments.

	Secondary ion	Form of addition	Stock solution (molar)	Secondary ion range of concentration ($mmol \cdot L^{-1}$)	Initial exposure medium ($mmol \cdot L^{-1}$)*
Body experiments	H^+	HCl	0.1	0.0001–1.0	pH 7, zero Ca^{2+}
	Ca^{2+}	$CaCl_2$	0.1	0–2.5	pH 7, zero Ca^{2+}
Gill and body experiments	H^+	HCl	0.1	0.0001–3.16	pH 7, zero Ca^{2+}
	H^+	H_2SO_4	0.05	0.0001–3.16	pH 7, zero Ca^{2+}
	Ca^{2+}	$CaCl_2$	0.1	0–11.20	pH 7, zero Ca^{2+}
	Al^{3+}	$Al_2(SO_4)_3$	0.1	0–1.0	pH 4, zero ca^{2+} and Al

*The initial exposure medium was 0.2 $mmol \cdot L^{-1}$ NaCl, initially adjusted to pH 7 with a few drops of 0.1 $mmol \cdot L^{-1}$ Trizma base (Sigma), to which secondary ions were added in the forms indicated.

5, 10, 15, 20, 25, or 30 s. Water samples (2 mL) were taken from the radiolabelled solution before each period of exposure for gamma counting and total Na^+ determination. At each time interval, at least seven fish were individually exposed.

After the final rinse in NaCl, fish were killed instantly by a blow to the head and blotted dry before taking a blood sample by cardiac puncture, using a freshly unpacked sterile syringe (Omnikan, 0.5 mL) for each fish. Blood sample volumes of between 50 and 300 μL, depending on the size of the fish, were counted for gamma activity. A second group of fish was similarly treated (using fresh solutions) for removal of the liver. This was achieved by clamping open the body cavity allowing liver removal without the organ contacting the fish exterior surfaces, and with minimal contact with other internal organs. A new scapel blade was used for each fish and all instruments and working surfaces were rinsed free of radiolabel between each sample. The livers were separately weighed and gamma counted.

In addition to the above procedures using solutions at pH 7, groups of fish were exposed to radiolabelled solutions (for 15 s) adjusted to pH 5 or 3 using H_2SO_4 or to radiolabelled solution containing 10 $mmol \cdot L^{-1}$ $CaCl_2$. These experiments determined the effects of H^+ or Ca^{2+} on Na^+ absorption into the blood over 15 s.

Gill and body surface adsorption experiments

These experiments were performed to show branchial as well as body surface Na^+ adsorption and the influences of other cations on the adsorption process. Free-swimming fish were immersed sequentially in the NaCl solutions previously described, spending 15 s in the radiolabelled solution. The fish were then killed as before, held with fine forceps to minimise disturbance to the body surface, and the branchial basket removed, a procedure normally completed in 20 s. Care was taken to avoid the fish touching anything other than the net (frequently washed nylon), scrupulously cleaned surgical gloves and instruments, or a frequently cleaned polythene bench surface. The carcass and branchial basket were separately weighed and then gamma counted.

The solutions used in these experiments were all made up by addition from stock solutions prepared in 0.2 $mmol \cdot L^{-1}$ NaCl (see Table 1) and examined for the influences of changes in H^+ or Ca^{2+} concentration on Na^+ adsorption by the gill and body surfaces.

Two further experiments were performed. The influence of ionic strength on Na^+ adsorption was examined. Ca^{2+} was added to 2.0 $mmol \cdot L^{-1}$ NaCl solutions (pH 7) for comparison with the data for 0.2 $mmol \cdot L^{-1}$ NaCl. Ionic valence effects were also examined. Fish were exposed to radiolabelled solution acidified to pH 4 and containing additions of Al (Table 1). This experiment was performed at pH 4 to ensure that the Al was predominantly in the Al^{3+} form (Booth et al. 1988), so that the influence of a trivalent ion on Na^+ adsorption could be compared with that of divalent and monovalent secondary ions.

Water samples (2 mL) were taken after each addition of Ca^{2+}, acid, or Al for ion analysis. Gamma activity of fish and water samples, as well as water Na^+ and Ca^{2+} concentrations, was determined as described. Aluminum was determined colourimetrically (Dougan and Wilson 1974).

Calculations, Statistics, and Units

The Na^+ adsorption for both the gill and body surface was calculated from the tissue count and the specific activity of the NaCl solutions. Tissues were counted for 15 min and corrected for background activity, always giving counts greatly in excess of 10 000 at the start of an experiment. However, since the experiments examined reductions in activity on the fish surface through the additions of secondary ions, in some experiments the total counts decreased to <10 000. Minimum total counts ranged between 4040 and 1000 above background when Na^+ adsorption was maximally depressed, which introduced random counting errors of about 3.2% when counts approached 1000. However, random counting error had an insignificant effect on standard errors of Na^+ adsorption within groups of fish, since experimental variation exceeded any variation due to random counting error. Therefore, variation between groups of fish could not be associated with low counts or random counting error.

Adsorption describes the number of molecules complexing with a particular surface (e.g. Pagenkopf 1983). Therefore, the units are concentration per unit area and in these experiments are nanomoles per square centimetre of gill or body area. Fish body area (Oikawa and Itazawa 1985) and gill area (Hughes and Al-Kadhomiy 1988) were calculated from the wet weights (W) of the whole fish, equating area with $719W^{0.664}$ and $316.71W^{0.932}$ respectively.

Sodium absorption into the blood or liver was calculated from the counts in the blood or liver divided by the specific activity of the bathing solution. Calculation of total Na^+ entry into the

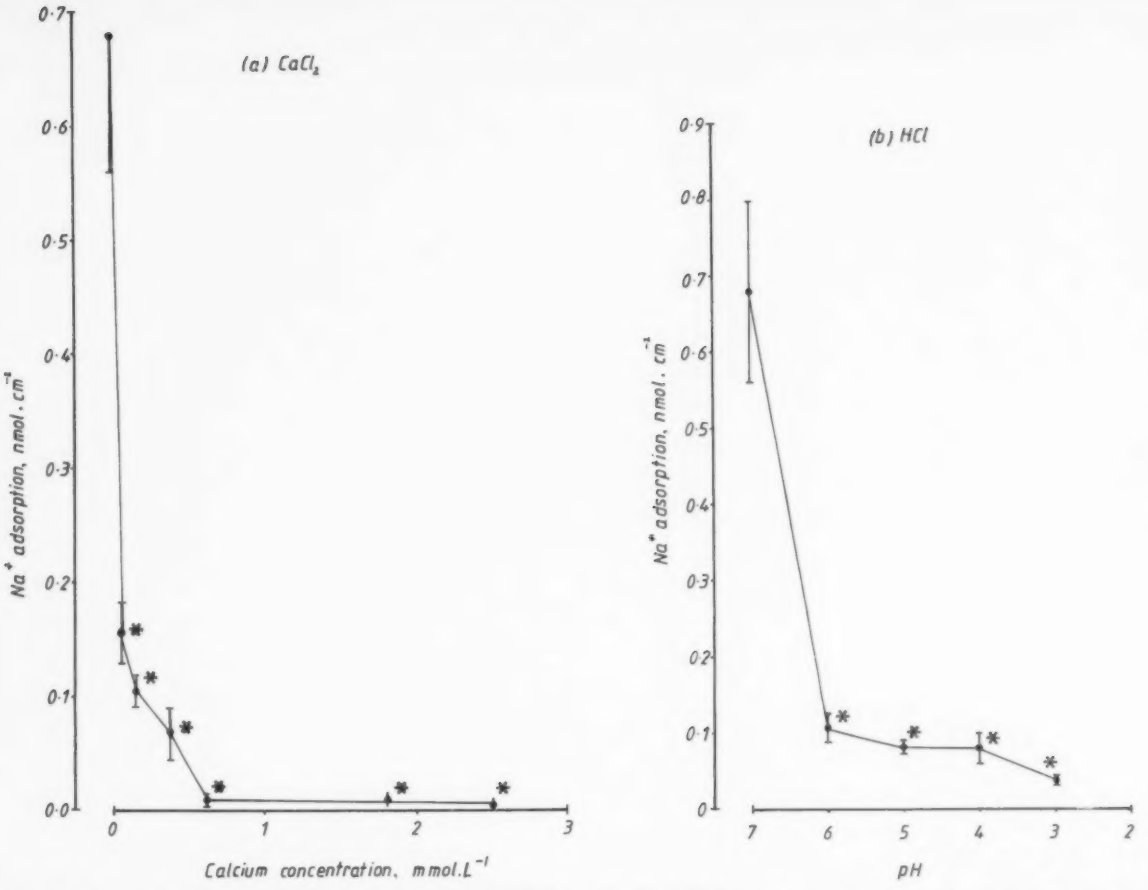

FIG. 1. Na^+ adsorption from 0.2 mmol·L^{-1} NaCl onto the body surface of immoblised trout (a) over a range of Ca^{2+} concentrations (added as $CaCl_2$) at pH 7 and (b) over a range of pH values (acidified with HCl) without Ca^{2+}. Points are means ± SE from seven fish. Asterisks indicate a significant difference from Na^+ absorption at pH 7, zero Ca^{2+} concentration. See text for details.

blood was achieved by multiplying Na^+ absorption per millilitre of blood by plasma volume (3.25% of the fish's wet weight; Gingerich and Pityer 1989). Extracellular fluid volume (ECFV) was not used to calculate total Na^+ entry into the fish because circulation times of 20–40 s for rainbow trout (Barron et al. 1987) suggest that Na^+ equilibrium within the ECFV was not achieved. Values for total Na^+ adsorption onto the surface of the fish are the sum of Na^+ adsorption onto the whole gill and whole body areas. Total counts on the gill were not corrected for radiolabel trapped in the plasma volume of the branchial basket, since plasma volume accounts for only 8% of gill weight (Gingerich and Pityer 1989). Total adsorption values are derived from the Na^+ adsorption per square centimetre of gill or body area. Total Na^+ accumulation is the total amount of Na^+ in/on the fish and thus is the sum of adsorption plus absorption.

The process of absorption may involve a time component and be expressed as a rate, e.g. micromoles per kilogram per hour. Estimations of Na^+ influx are derived from the total Na^+ entry into the blood in 15 s and fish weight.

Data were tested by analysis of variance (ANOVA) with a significance level of $P = 0.05$ and are expressed as means ± standard errors.

Results

Experiments with Immobilised Fish

The adsorption of Na^+ by the body surface alone (excluding the gills) was influenced by both water Ca^{2+} concentration and pH (Fig. 1). Sodium adsorption was reduced significantly from 0.68 to 0.008 nmol·cm^{-2} with increases in Ca^{2+} concentration to 2.50 mmol·L^{-1}. Lowering water pH from 7 to 3 reduced body surface Na^+ adsorption from 0.67 to 0.045 nmol·cm^{-2}. The greatest reductions in Na^+ adsorption occurred below 0.6 mmol Ca^{2+}·L^{-1} and between pH 7 and 6.

Absorption to Internal Tissues

Free-swimming fish absorbed detectable levels of the external Na^+ (pH 7, zero Ca^{2+}) within 30 s (Fig. 2). Sodium absorption into the blood remained at 17 nmol·mL^{-1} for the first 15 s, increasing rapidly thereafter, perhaps reflecting the time it takes radiolabel to cross the gills, spread through the blood compartment, and circulate to the heart. Total counts in the radiolabelled solution ranged between 418 358 and 280 194 during the experiment, giving specific activities of about 4200 cpm·$nmol^{-1}$.

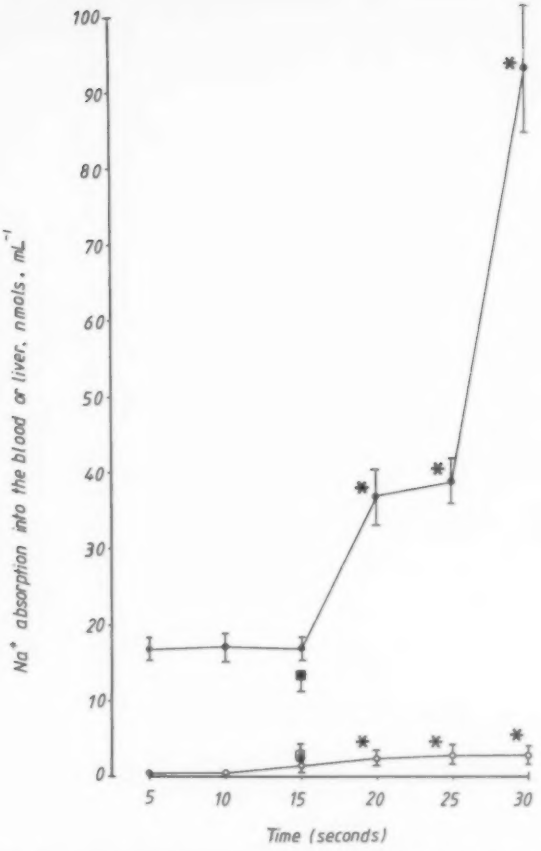

FIG. 2. Appearance of external Na^+ in the blood (●) or livers (○) of free-swimming trout exposed to 0.2 mmol·L^{-1} NaCl solution for various times at pH 7 in the absence of Ca^{2+} and after 15 s of exposure to NaCl at pH 3 (□) or pH 5 (■) (acidified with HCl, zero Ca^{2+}), or containing 10 mmol Ca^{2+}·L^{-1} (▲) (added as $CaCl_2$ at pH 7). Points are means ± SE from six fish. Asterisks indicate a significant difference from the 5-s means (pH 7, zero Ca^{2+} data only). See text for details.

Sodium was also absorbed into the livers of free-swimming fish over 30 s (Fig. 2). However, Na^+ entry into the liver at 30 s was a maximum of 2.3 nmol·g wet weight^{-1} compared with 93.6 nmol·mL^{-1} in the blood at this time. Thus, Na^+ entry into the liver was an order of magnitude lower than that into the blood and may have been completely derived from blood contained within the livers. Therefore, most of the Na^+ entering the fish in such short experiments is contained in the blood. Total counts in the radiolabelled solution during the liver removal experiment ranged from 683 164 to 490 244, giving specific activities of about 6000 cpm·nmol^{-1}.

The addition of acid to the external solution (using 15-s exposures) reduced Na^+ absorption into the blood significantly from 17.17 to 3.46 nmol·mL^{-1} at pH 3, although a decrease to 13.58 nmol·mL^{-1} at pH 5 was not significant (Fig. 2). Calcium additions (using a 15-s exposure) also significantly reduced Na^+ absorption into the blood from 17.17 to 2.77 nmol·mL^{-1} (Fig. 2). Total counts in the radiolabelled solution ranged from 342 660 to 100 600 using acid or Ca^{2+} additions, giving specific activities of about 2200 cpm·nmol^{-1}.

When using a 15-s exposure period to radiolabelled solution (pH 7, zero Ca^{2+}), 84% of the Na^+ taken up remained on the fish surface (Table 2). In addition to reducing Na^+ adsorption to the fish surface, acid or Ca^{2+} also reduced Na^+ entry into the blood so that over 80% of the Na^+ taken up remained on the exterior surface. Thus, in a range of experimental conditions, using a 15-s exposure time, surface effects predominated (Table 2).

Gill and Body Surface Adsorption Experiments

Sodium adsorption by both the gill and body surfaces of free-swimming fish was decreased as pH was reduced (Fig. 3). Additions of acid, as either HCl or H_2SO_4, reduced both gill and body Na^+ adsorption nmol·L^{-1} to 0.1 or less (Fig. 3). Furthermore, Na^+ adsorption at all pH values was not significantly different for HCl compared with H_2SO_4 additions, $Al_2(SO_4)_3$ indicating the absence of anion effects (Fig. 3). Additions of $Al_2(SO_4)_3$ at pH 4 had no significant effect on either gill or body Na^+ adsorption (Fig. 4).

Changes in water Ca^{2+} concentration also influenced Na^+ adsorption by the body and gill surface of free-swimming fish (Fig. 5a). Additions of 0–11.2 mmol Ca^{2+}·L^{-1} reduced Na^+ adsorption from 0.54 to 0.1 nmol·cm^{-2} on the body surface and from 0.35 to 0.07 nmol·cm^{-2} on the gill using 0.2 mmol·L^{-1} NaCl solutions (Fig. 5a); this effect was abolished at higher ionic strength in the presence of 2 mmol·L^{-1} NaCl (Fig. 5b).

Na^+ adsorption by the body was generally greater than by the gill in the experiments with free-swimming fish (Fig. 3 and 5).

Discussion

Evidence for Surface Adsorption

Experiments with immobilised fish

Early experiments on fish osmoregulation demonstrated that fish skin generally has a negligible role in ion transport (Krogh 1937). Thus the body surface provides a good model for examining adsorption phenomena in the absence of active processes. Figure 1 demonstrates two important aspects of adsorption: (1) Na^+ adsorption onto the fish surface can occur in the absence of active uptake, and is therefore not "driven" by active transport, and (2) Na^+ adsorption can be influenced by the presence of other ions in the bathing media, such as H^+ or Ca^{2+}.

Experiments with free-swimming fish

Quantifying adsorption phenomena is more difficult when active uptake or absorption occurs on the same surface, as in the case of the gills. The experimental protocol was not able to completely exclude active uptake because Na^+ appeared in the blood within seconds (Fig. 2). However, by choosing a suitable exposure period to the radiolabelled solution, minimum uptake compared with adsorption could be achieved.

Using an exposure period of 15 s, sodium entry into the blood remained low (Table 2). Sodium entry into the livers was much lower than the blood (Fig. 2), and the short circulation time for rainbow trout (about 40 s; Barron et al. 1987) indicates that most of the radiolabel would be in the blood rather than fully distributed in the ECFV. Thus, total Na^+ entry into the fish could be calculated provided plasma volume was known (Table 2). The apparent sudden increase in absorption at 20 s

TABLE 2. Na^+ adsorption onto the fish surface (gill plus body) compared with Na^+ absorption into the blood over 15 s. Medium was 0.2 mmol·L^{-1} NaCl without acid or Ca^{2+} additions (pH 7, zero Ca^{2+}), in the presence of HCl at pH 5 or 3 (zero Ca^{2+}), or with 10 mmol·L^{-1} $CaCl_2$ (pH 7). Plasma volumes were calculated assuming the plasma occupies 3.25% of the fish's wet weight (Gingerich and Pityer 1989). Total Na^+ entry (absorption) into the plasma was calculated from the data in Fig. 2 and plasma volumes. Total Na^+ adsorption onto the fish surface values were derived from Fig. 3b (pH 7, 5, or 3, zero Ca^{2+}) and 5a (pH 7, 10 mmol Ca^{2+}·L^{-1}) and the total surface area of both gill and body. See text for details.

Medium	Mean fish weight (g)	Plasma volume (mL)	Total Na^+ entry into plasma (absorption) (nmol)	Total Na^+ adsorption onto fish surface (gill + body) (nmol)	Total Na^+ accumulation by fish (absorption + adsorption) (nmol)	Accumulated Na^+ on fish surface (%)
pH 7, zero Ca^{2+}	4.5	0.146	2.510	14.118	16.698	84.96
pH 5, zero Ca^{2+}	4.0	0.130	1.765	8.641	10.406	83.03
pH 3, zero Ca^{2+}	4.0	0.130	0.449	1.854	2.303	80.50
pH 7, 10 mmol Ca^{2+}·L^{-1}	4.0	0.130	0.360	2.726	3.086	88.33

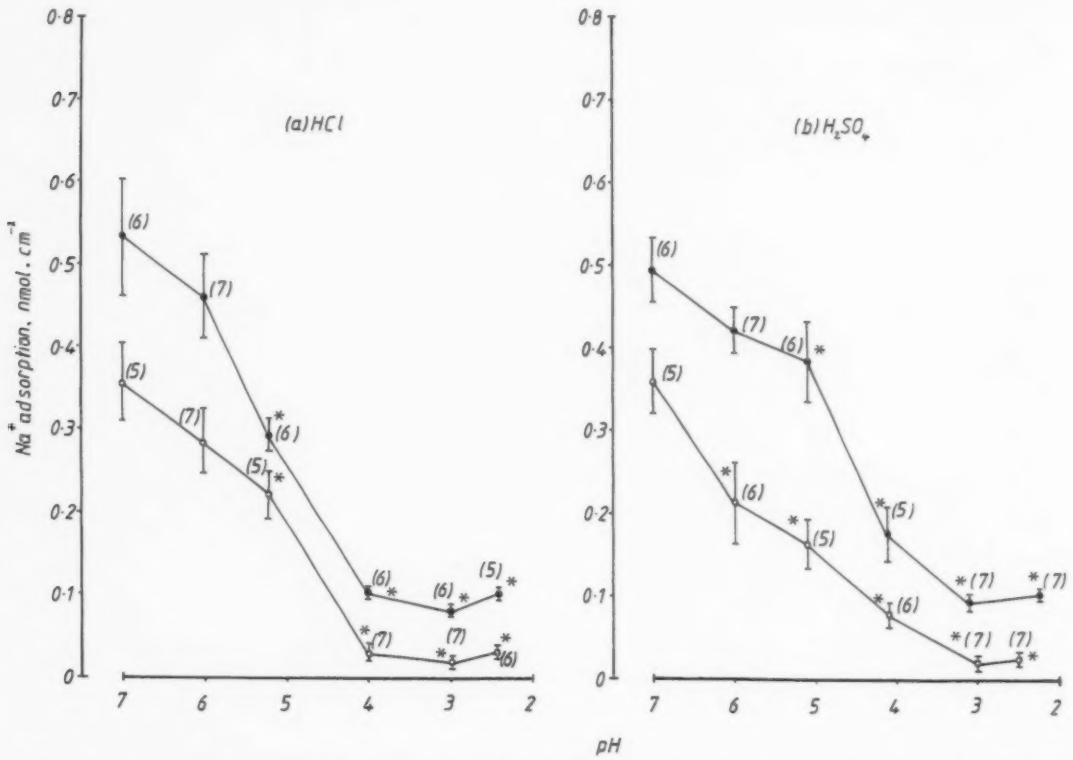

FIG. 3. Na^+ adsorption from 0.2 mmol·L^{-1} NaCl onto the body (●) and gill surface (○) of free-swimming trout over a range of pH values without Ca^{2+}. (a) HCl; (b) H_2SO_4. Points are means ± SE; number of observations are in parentheses. Asterisks indicate a significant difference from the pH 7 mean. See text for details.

(Fig. 2) could be a function of circulation time. Blood sampling took about 30–60 s, thus allowing blood to circulate only once. Exposure to radiolabel for 20–30 s in addition to blood sampling time may allow the blood to pass through the gills two or three times, accumulating radiolabel each time. Since blood samples were taken at a point some distance from the site of radiolabel entry into the circulation, any radioactivity present in samples is likely to be representative of the whole plasma. Therefore, the sudden increase in absorption at 20 s may not be attributed to a sudden increase in influx rate.

Sodium entry into the fish was lower than apparent adsorption onto the fish's exterior, in a range of experimental conditions, suggesting that adsorption phenomena account for over 80% of the total Na^+ sorption in/on the fish over short time periods (Table 2).

Relationship between Adsorption and Branchial Na^+ Influx

The movement of waterborne Na^+ across the gills into the blood of freshwater salmonids is a key process in fish osmoregulation. However, our understanding of the factors which influence the ability of Na^+ to reach the sites of ionic capture and uptake is limited. Clearly, Na^+ must be adsorbed onto the gill surface before influx can occur. This is supported by cor-

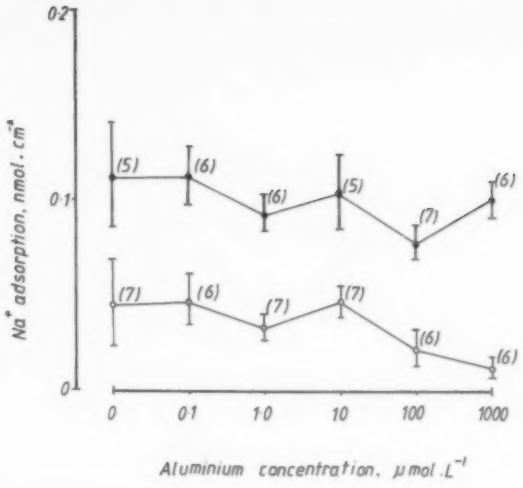

FIG. 4. Na^+ adsorption from 0.2 mmol·L^{-1} NaCl onto the body (●) and gill surface (○) of free-swimming trout at pH 4.0 (H_2SO_4) over a range of Al (as $Al_2(SO_4)_3$) concentrations without Ca^{2+}. Points are means ± SE; number of observations are in parentheses. See text for details.

relations between Na^+ adsorption and entry into the blood, as well as previously determined values for Na^+ influx (Tables 2 and 3). Reduced solution pH resulted in decreased Na^+ entry into the blood (Table 2; Fig. 2), Na^+ influx (Table 3), and adsorption onto the fish surface (Fig. 3; Table 2). Thus, adsorption and influx are linked, and factors which effect Na^+ adsorption will ultimately effect Na^+ influx.

The characteristics of Na^+ adsorption observed (Fig. 3, 4, and 5a) reflect known effects of H^+, Al, and Ca^{2+} on branchial Na^+ net uptake rates. Both H^+ and Ca^{2+} reduce Na^+ influx rates (McWilliams and Potts 1978; Booth et al. 1988; Goss and Wood 1988), while Al has a negligible effect at pH 4, since Na^+ influx already is substantially inhibited by H^+ (Booth et al. 1988). Similarly, Na^+ adsorption to the gill surface, and thus Na^+ availability to the branchial transport mechanism, is reduced by increases in H^+ or Ca^{2+} levels (Fig. 3 and 5a) whereas Al at pH 4 has a minimal effect (Fig. 4). Therefore, adsorption effects may make a significant contribution to the apparent inhibition characteristics of Na^+ influx in the presence of ions such as H^+ or Ca^{2+}.

The Na^+ influx rates (Table 3) were calculated to illustrate the relationship between adsorption and influx. Despite the short duration of influx measurements, the values from this study correspond with those previously determined for salmonids in similar conditions (Table 3). Perhaps the method here could be generally useful for rapid determination of Na^+ influx values in kinetic studies.

Physicochemical Influences on Adsorption

There are several physicochemical factors which may influence the adsorption process: (1) competition between ions for a limited number of surface binding sites, (2) the total number and type of surface binding sites (Pagenkopf 1983), (3) solution ionic strength and ionic mobility (Robinson and Stokes 1965), and (4) unstirred layer formation (Pedley 1983). The results (Fig. 3 and 5) suggest that all four factors may influence Na^+ binding to the gill surface. For example, the influence of Ca^{2+} on branchial Na^+ adsorption (Fig. 5a) was abolished with a 10-fold increase in Na^+ concentration to 2.0 mmol·L^{-1} (Fig. 5b). Increased competition between Na^+ and Ca^{2+} for cell surface ligands may partly explain this effect. Cusimano et al. (1986) suggested a similar explanation for the ameliorative effects of H^+ on Zn toxicity.

Sodium adsorption onto the body is generally greater than onto the gill (Fig. 3 and 5a) and may be related to the presence of more metal-binding ligands on the body surface compared with the branchial epithelium. Mucus provides a source of metal-binding ligands (Handy et al. 1989) on the body surface. However, the gills of unstressed rainbow trout have a limited or absent mucus coat (Handy and Eddy 1989, 1991), suggesting that the gill has fewer surface binding sites compared with the body. Despite this difference the general trends in the adsorption on the gill and body were similar, probably because cation binding to a range of biological surfaces is achieved with only a few types of fixed anionic residues (Campbell and Stokes 1985).

Ionic strength and mobility may also be important. Additions of Ca^{2+} to dilute solutions will increase solution ionic strength and ultimately reduce the mobility of all ions in the solution (Robinson and Stokes 1965), perhaps slowing the adsorption process. Thus, additions of Ca^{2+} (Fig. 5a) could cause reduced Na^+ adsorption via ionic mobility effects. In addition, the relative mobility of ions within a solution may be important. For example, H^+ is the most mobile ion in solution, while Ca^{2+} with a higher "charge density" is relatively immobile (Robinson and Stokes 1965). Lower H^+ compared with Ca^{2+} concentrations (0.1 and 3.7 mmol·L^{-1}; Fig. 3a and 5a, respectively) are required to achieve maximal reductions in Na^+ adsorption. Thus the more mobile H^+ is the more effective ion at reducing Na^+ adsorption within a solution. Clearly, water quality influences Na^+ adsorption onto the gill surface and ultimately the supply of Na^+ for active transport, perhaps also the ion selectivity normally associated with gill membranes.

Unstirred layer formation may also affect adsorption. Some evidence for this lies in differences between adsorption by immobilised and free-swimming fish. Swimming activity may slough mucus and increase medium stirring adjacent to the body surface, resulting in reduced unstirred layer thickness (Pedley 1983). This may result in reduced adsorption onto the surface of free-swimming fish. The initial body adsorption values (zero Ca^{2+}, pH 7) for free-swimming fish (Fig. 3a and 5a) appear marginally lower than those for immobilised animals (Fig. 1), although the differences are not statistically significant. Clearly, effects of an unstirred layer are worthy of more investigation.

Ionic adsorption by the gill surface is probably not controlled by one physicochemical process. A net movement of Na^+ from the water to the gill surface must ultimately occur to fuel Na^+ influx (in the absence of ion recycling at the cell surface). The influence of 2.0 compared with 0.2 mmol·L^{-1} NaCl on the Ca^{2+} effect (Fig. 5), and the absence of an Al effect in the presence of H^+ (pH 4, Fig. 4), supports the idea of simple ion exchange at the gill surface ligands (Pagenkopf 1983). However, the notable reduction in Na^+ adsorption due to micromolar increases in H^+ or Ca^{2+} correlates with H^+ and Ca^+ effects on overall solution ionic strength and ionic mobility, suggesting that Na^+ is "restrained" in the external medium by other ions. Thus, both ion exchange at the cell surface and solution chemistry must be considered.

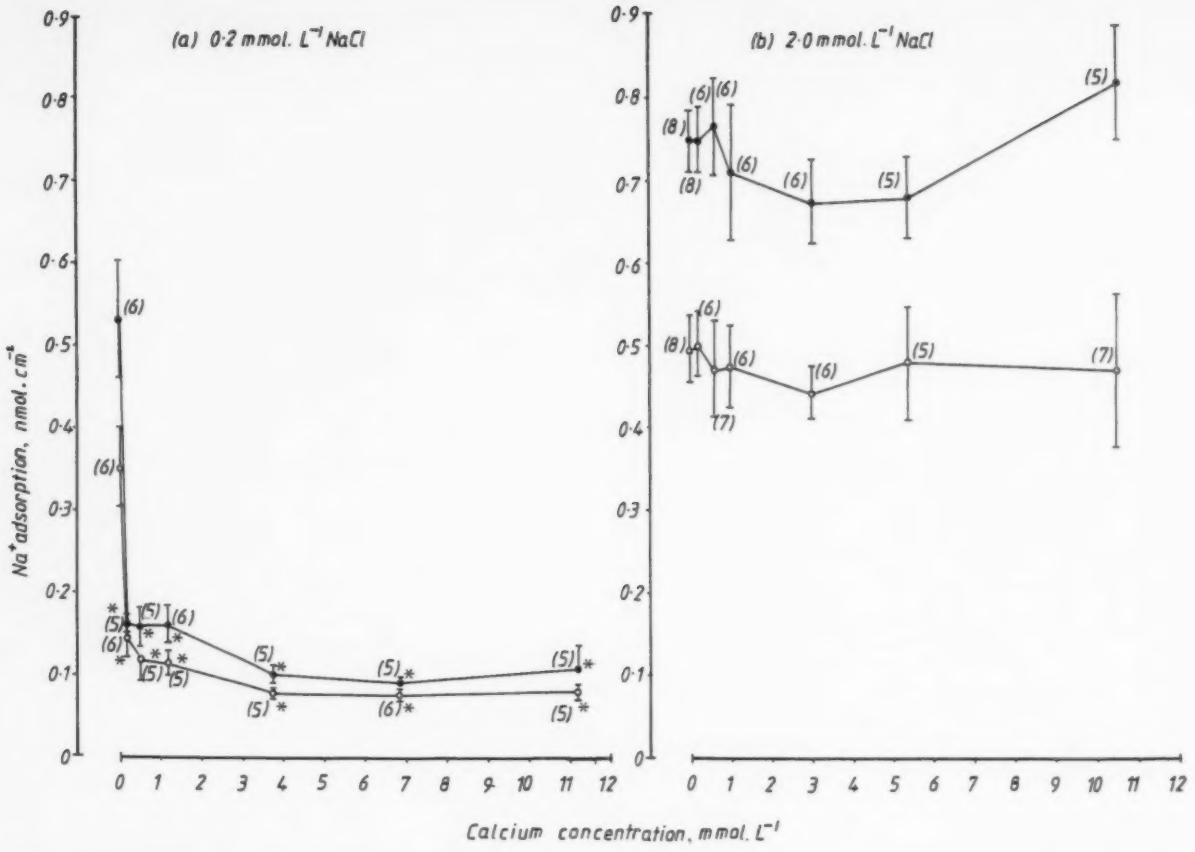

FIG. 5. Na^+ adsorption onto the body (●) and gill surface (○) of free-swimming trout over a range of Ca^{2+} concentrations (added as $CaCl_2$) at pH 7 using (a) 0.2 and (b) 2.0 mmol·L^{-1} NaCl solutions. Points are means ± SE; number of observations are in parentheses. Asterisks indicate a significant difference from the zero Ca^{2+} concentration mean. See text for details.

TABLE 3. Estimation of Na^+ influx determined from Na^+ absorption into the blood over 15 s compared with reported values. Na^+ influx values in this study were determined from the total Na^+ entry into the fish (Table 2) over 15 s. Cited influx values were determined over several hours, generally using free-swimming fish. Ca^{2+} and Na^+ concentrations are in mmol·L^{-1}. See text for details.

Na^+ influx value (μmol·kg^{-1}·h^{-1})	Species	Experimental conditions	Reference
133.8	*Oncorhynchus mykiss*	pH 7, zero Ca^{2+}, 0.2 Na^+	This study
130	*Salmo trutta*	pH 7, 0.5 Ca^{2+}, 0.25 Na^+	McWilliams and Potts 1978
150	*Salvelinus fontinalis*	pH 6.5, 0.02–0.40 Ca^{2+}, 0.03–0.5 Na^+	Booth et al. 1988
140	*Oncorhynchus mykiss*	Circumneutral pH, 1.1 Ca^{2+}, 0.2 Na^+	McDonald and Rogano 1986
105.9	*Oncorhynchus mykiss*	pH 5, zero Ca^{2+}, 0.2 Na^+	This study
60	*Salmo trutta*	pH 5, 0.5 Ca^{2+}, 0.25 Na^+	McWilliams and Potts 1978
80–120	*Salvelinus fontinalis*	pH 5.2–4.8, 0.02–0.40 Ca^{2+}, 0.03–0.5 Na^+	Booth et al. 1988
26.9	*Oncorhynchus mykiss*	pH 3, zero Ca^{2+}, 0.2 Na^+	This study
25	*Salmo trutta*	pH 4, 0.5 Ca^{2+}, 0.25 Na^+	McWilliams and Potts 1978
50	*Salvelinus fontinalis*	pH 4.4, 0.02–0.46 Ca^{2+}, 0.03–0.50 Na^+	Booth et al. 1988
21.60	*Oncorhynchus mykiss*	pH 7, 10 Ca^{2+}, 0.2 Na^+	This study

Environmental Implications

Sodium adsorption by the gill and body surface are clearly influenced by micromolar changes in H^+ or Ca^{2+} concentration (Fig. 1, 3, and 5a). Decreases in solution pH between pH 7 and 5, or increases in Ca^{2+} concentration up to 0.5 mmol·L^{-1} (Fig. 3 and 5), particularly reduced both gill and body Na^+ adsorption. Therefore in dilute freshwaters, where water pH and Ca^{2+} concentration may fluctuate (Sadler and Turnpenny 1986; McCahon and Pascoe 1989), the amount of Na^+ available to the fish surface may also be dramatically influenced. In

soft waters of low pH, reduced branchial Na^+ adsorption may ultimately result in reduced Na^+ influx and ionoregulatory disturbance. Moreover, because the adsorption process is so sensitive to slight changes in water quality, normal environmental fluctuations may have considerable effects on Na^+ availability to the gills.

In their natural environment, salmonids may constantly experience changes in bulk water solute chemistry. For example in one study, water pH varied between pH 7 and 4, while Ca^{2+} levels ranged between 0.025 and 0.67 $mmol \cdot L^{-1}$ in upland streams (Sadler and Turnpenny 1986). Thus, salmonids may normally experience fluctuations in Na^+ adsorption and therefore Na^+ availability to the gill surface. Although opercular water in contact with the gill surface acts as a buffer reducing apparent bulk water pH fluctuations (Playle and Wood 1989), Na^+ adsorption and ultimately Na^+ uptake may require constant readjustment of branchial functions, adding an as yet unknown cost to ionic regulation. Clearly, the Na^+ adsorption process may be important in Na^+ uptake homeostasis under normal environmental conditions.

In conclusion, the availability of Na^+ to the gill cell apical membrane and thus to the ion transporting mechanism is influenced by the process of adsorption which is sensitive to slight fluctuations in water pH and Ca^{2+} concentration. The characteristics of Na^+ adsorption by the gill surface correlate with observed disturbances to Na^+ influx during toxic metal exposure, suggesting that Na^+ adsorption effects are important in branchial ionoregulatory disturbances. The sensitivity of the adsorption process to slight variations in water quality indicates that Na^+ availability to the gill will vary during normal fluctuations in environmental conditions as part of the ordinary ionoregulatory process. This paper also illustrates the rapidity of Na^+ movement across the gill epithelium and the consequent difficulties in separating surface phenomena from active processes. The complex structure of the gill, the variety of ventilatory responses to environmental conditions, and the status of the branchial unstirred layer may also affect ionic capture. These factors are currently under investigation.

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References

BARRON, M. G., B. D. TARR, AND W. L. HAYTON. 1987. Temperature dependence of cardiac output and regional blood flow in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 31: 735–744.

BOOTH, C. E., D. G. MCDONALD, B. P. SIMONS, AND C. M. WOOD. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1563–1574.

BROWN, D. J. A. 1983. Effects of calcium and aluminum concentrations on the survival of brown trout (*Salmo trutta*) at low pH. Bull. Contam. Toxicol. 30: 582–587.

CAMPBELL, P. G. C., AND P. M. STOKES. 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. 42: 2034–2049.

CUSIMANO, R. F., D. F. BRAKKE, AND G. A. CHAPMAN. 1986. Effects of pH on the toxicities of cadmium, copper, and zinc to steel head trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 1497–1503.

DOUGAN, W. K., AND A. L. WILSON. 1974. The absorptiometric determination of aluminium in water. A comparsion of some chromogenic reagents and the development of an improved method. Analyst 99: 413–430.

GINGERICH, W. H., AND R. A. PITYER. 1989. Comparison of whole body and tissue blood volumes in rainbow trout (*Salmo gairdneri*) with ^{125}I bovine serum albumin and ^{51}Cr-erythrocyte tracers. Fish Physiol. Biochem. 6: 39–47.

GOSS, G. G., AND C. M. WOOD. 1988. The effects of acid and acid/aluminum exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout, *Salo gairdneri*. J. Fish Biol. 32: 63–76.

HANDY, R. D., AND F. B. EDDY. 1989. Surface absorption of aluminium by gill tissue and body mucus of rainbow trout, *Salmo gairdneri*, at the onset of episodic exposure. J. Fish Biol. 34: 865–874.

1991. The absence of mucus on the secondary lamellae of unstressed rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Biol. 38: 153–155.

HANDY, R. D., F. B. EDDY, AND G. ROMAIN. 1989. *In vitro* evidence for the ionoregulatory role of rainbow trout mucus in acid, acid/aluminium and zinc toxicity. J. Fish Biol. 35: 737–747.

HUGHES, G. M., AND N. K. AL-KADHOMIY. 1988. Changes in scaling of respiratory systems during the development of fishes. J. Mar. Biol. Assoc. U.K. 68: 489–498.

KROGH, A. 1937. Osmotic regulation in freshwater fishes by active absorption of chloride ions. Z. Vgl. Physiol. 24: 656–666.

MAZEAUD, M. M., AND F. MAZEAUD. 1981. Adrenergic responses to stress in fish, p. 49–75. *In* A. D. Pickering [ed.] Stress and fish. Academic Press, London.

MCCAHON, C. P., AND D. PASCOE. 1989. Short term experimental acidification of a Welsh stream: toxicity of different forms of aluminum at low pH to fish and invertebrates. Arch. Environ. Contam. Toxicol. 18: 233–242.

MCDONALD, D. G. 1983. The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. I. Branchial and renal net ion and H^+ fluxes. J. Exp. Biol. 102: 123–140.

MCDONALD, D. G., AND M. S. ROGANO. 1986. Ion regulation by the rainbow trout, *Salmo gairdneri*, in ion-poor water. Physiol. Zool. 59: 318–331.

MCWILLIAMS, P. G., AND W. T. W. POTTS. 1978. The effects of pH and calcium concentrations on gill potentials in the brown trout, *Salmo trutta*. J. Comp. Physiol. 126: 277–286.

OIKAWA, S., AND Y. ITAZAWA. 1985. Gill and body surface areas of the carp in relation to body mass, with special reference to the metabolism–size relationship. J. Exp. Biol. 117: 1–14.

PAGENKOPF, G. K. 1983. Gill surface interaction model for trace metal toxicity to fishes: role of complexation, pH, and water hardness. Environ. Sci. Technol. 17: 342–347.

PEDLEY, T. J. 1983. Calculation of unstirred layer thickness in membrane transport experiments: a survey. Q. Rev. Biophys. 16: 115–150.

PICKERING, A. D., T. G. POTTINGER, AND P. CHRISTIE. 1982. Recovery of brown trout, *Salmo trutta* L., from acute handling stress: a time course study. J. Fish Biol. 20: 229–244.

PLAYLE, R. C., AND C. M. WOOD. 1989. Water chemistry changes in the gill microenvironment of rainbow trout: experimental observations and theory. J. Comp. Physiol B 159: 527–537.

REID, S. D., AND D. G. MCDONALD. 1988. Effects of cadmium, copper, and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45: 244–253.

ROBINSON, R. A., AND R. H. STOKES. 1965. Electrolyte solutions. 2nd ed. revised. Butterworths, London. 118 p.

SADLER, K., AND A. W. H. TURNPENNY. 1986. Field and laboratory studies of exposures of brown trout to acid waters. Water Air Soil Pollut. 30: 593–599.

SPRY, D. J., AND C. M. WOOD. 1985. Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. Can. J. Fish. Aquat. Sci. 42: 1332–1341.

Longevity, Body Size, and Growth in Anadromous Brown Trout (*Salmo trutta*)

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Longevity in 25 populations of anadromous brown trout (*Salmo trutta*) showed a significant trend with increasing life span at latitudes of 58–70°N in Norway, with the largest change from 58 to 60°N. Moreover, longevity was negatively correlated with temperature and growth rate in freshwater and at sea. Body size was negatively correlated with water temperature and growth rate in freshwater, but not with latitude or water temperature and growth rate at sea. Thus, conditions influencing development and metabolic rates in fresh water seem more important than conditions in the sea in determining variation in longevity and body size of anadromous brown trout. Our results support the hypothesis that interpopulation variation in longevity and body size is influenced by rate of metabolism, chiefly influenced by ambient water temperature.

On a constaté que la longévité mesurée dans 25 populations anadromes de truite brune (*Salmo trutta*) présente une tendance significative, sa valeur augmentant entre 58 et 70° de latitude N en Norvège, le changement le plus marqué s'observant entre 58 et 60°. En outre, la longévité est en corrélation négative avec la température et le taux de croissance en eau douce et en mer. La taille du poisson est en corrélation négative avec la température de l'eau et le taux de croissance en eau douce, mais non avec la latitude, ni avec la température de l'eau et le taux de croissance en mer. Ainsi, les conditions qui influent sur le développement et le métabolisme en eau douce semblent importer davantage que les conditions en mer lorsqu'on veut déterminer la variation de la longévité et de la taille chez la truite brune anadrome. Nos résultats viennent étayer l'hypothèse selon laquelle la variation de la longévité et de la taille entre des populations dépend du métabolisme, qui dépend lui-même surtout de la température de l'eau ambiante.

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There is a strong positive correlation between the size of an organism and its longevity (Bonner 1965; Calder 1984). A possible reason is that as size increases, the rate of metabolism per unit weight decreases, leading to increased longevity (Fenchel 1974; Peters 1983). Among poikilotherms, the metabolic rate differs among climatic zones and increases with the temperature within these zones (Brett and Groves 1979). When the level of standard metabolism has been established for a species, the use of 2.3 as a multiplier would provide a near approximation for temperature effects within the span of ± 10°C (Beamish 1964; Brett 1964). Fish living in colder environments should therefore have longer lives than those living in warmer water. This relationship should be especially pronounced within species, resulting in latitudinal clines in longevity. So far, however, there is little evidence for such a within-species variation, with the work on American shad (*Alosa sapidissima*) as the exception (Leggett and Carscadden 1978; Glebe and Leggett 1981).

Bergmann's rule states that within a species, races from cooler climates tend to be larger than races living in warmer parts of the range (Mayr 1956). Among poikilotherm vertebrates, body size may in many cases increase with latitude in accordance with Bergmann's rule (Ray 1960; Lindsey 1966). This size trend cannot be explained by heat conservation, as metabolic rates of these animals are chiefly influenced by variation in ambient temperature. In fishes, this latitudinal trend in body size may be attributed to delayed age at sexual maturity as a response to reduced growth rate and mortality at low water temperature (Alm 1959; Jonsson 1977; Pauly 1980; Thorpe 1986).

Within stocks, fast-growing fish usually mature younger than slow growers (Jonsson et al. 1984; Thorpe 1986). However, they may also die earlier due to high energetic costs associated with reproduction at a young age (Reznik and Endler 1982). The relationship among stocks within a species is, however, less well documented.

Based on a regional study of 25 populations of anadromous brown trout (*Salmo trutta*) situated from 58 to 70°N in Norway, we tested the hypothesis that the longevity and body size of brown trout are independent of latitude, temperature in freshwater and the sea, and growth rate of the fish.

Methods

Brown Trout Life History

Anadromous brown trout spawn in streams of Norway during September and November. Streams, rivers, and lakes serve as nurseries for the young (parr) from 1 to 6 yr of age (L'Abée-Lund et al. 1989; Borgstrøm and Heggenes 1989). Some parr (0.6–62%), especially males, mature sexually and become residents (Jonsson 1985; Dellefors and Faremo 1988; L'Abée-Lund et al. 1990). The fish smolt during spring, usually at a length between 10 and 23 cm (L'Abée-Lund et al. 1989), and migrate into coastal waters where they feed during summer (Jensen 1968; Jonsson 1985; Berg and Berg 1987). During autumn, both immature and mature fish return to their home river where they spend the winter (Nordeng 1977). However, some straying between rivers may occur (Pratten and Shearer 1983; Berg and Berg 1987). This annual migration to and from the sea is repeated each year for the rest of their lives (Jonsson 1985; Berg and Jonsson 1990).

Sampling

Anadromous brown trout were investigated in 25 Norwegian rivers (Fig. 1). Fish were sampled during the period 1976–89 with electrical fishing gear, gill nets, and by angling (Table 1). Fish were measured (millimetres) when laying in natural position (natural tip length; Ricker 1979) and classified as mature when the gonads were developed for spawning in autumn (Dahl 1917). Ages were determined from scales (Jonsson 1976, 1985). Anadromous brown trout scales seldom develop spawning marks such as those seen in Atlantic salmon (*Salmo salar*) scales, and when they do, the annuli are not erased and are still recognizable. Lengths at age were estimated by back-calculation from scales assuming that the relationship between length of the scale and that of the fish was linear (Lea 1910; Jonsson 1985). In this paper, we only used differences between two successive ages to illustrate growth increments, avoiding errors in back-calculation that this method otherwise could cause (see Carlander 1980). Anadromous brown trout were usually distinguished from coexisting resident brown trout by their larger body size. In addition, anadromous brown trout were recognized by their scale pattern, which showed a contrast between river and sea growth (see e.g. Frost and Brown 1967; Fahy 1985, cited in Wootton 1990).

Definitions

Separate age distributions of males and females were estimated for each river. The longevity of each sex was defined as the age at the 90th percentile of the age distribution. In fishes, growth rate decreases after sexual maturity (Nikolsky 1963). Thus, body size is defined as the length at first maturity. Determination of age at sexual maturity was based on reading scales (Dahl 1910). With sexual maturity, the annual length increment is reduced by about 50% compared with immature fish of the same age, and even more compared with a fish's own growth in the previous year (Jonsson 1985). Furthermore, especially in females the annuli formed following spawning are very distinct (see e.g. Fahy 1985, cited in Wootton 1990).

The length increment of brown trout during the second year in the river was used to describe parr growth in relation to river temperature. The length increment during the first year in the river was omitted due to uncertainty when embryos started to grow. The length increment during the third year could not be used because at this age, most brown trout in southern Norwegian rivers had already smolted (mean smolt age approximately 2.2 yr; L'Abée-Lund et al. 1989). The length increment during the first summer at sea was used to describe the growth pattern at age within each population in relation to ambient sea temperature. The length increment during the second, third, and later summers at sea could not be used because sexual maturation would bias the results by underestimating the potential length increment of anadromous brown trout at sea (cf. Jensen 1968; Jonsson 1985).

Environmental Parameters

We designated the latitude of each river by the latitude of its mouth. River temperatures were collected by the Norwegian Water and Electricity Board, Hydrological Department, in the lower part of the rivers. The river temperatures used in the present study are means of recordings made over periods from 2 to 15 yr. The temperature was measured at least every second day, and in most cases at 08:00 and 16:00. The period of the year when the water temperature exceeded 4°C was used to calculate the degree-days of growth. This was done by averaging water temperature over each three 10-d interval within each month, summing the intervals with temperature exceeding 4°C, and multiplying by number of days. The reason for selecting 4°C is that growth of brown trout commences at this temperature (Elliott 1975).

The length of the annual feeding period for smolts and older trout in the sea at various latitudes was determined by observations of migrations of anadromous brown trout in the Rivers Sandvikselva (59°53′N, 10°30′E; Rosseland 1965), Vosso (Jonsson 1985), Istra (Jensen 1968), Vardneselva (69°10′N, 17°30′E; Berg and Berg 1987), and Tana (70°22′N, 28°13′E; Niemelä and McComas 1985) as well as unpublished observations from the Rivers Imsa and Halselva (70°02′N, 22°53′E) (Fig. 1; Table 1). The migration pattern was recorded by traps in four of these rivers (Imsa, Istra, Vardneselva, and Halselva). The annual feeding period at sea was used when estimating number of degree-days during which smolts and older trout were at sea. The sea temperatures are means for each three 10-d interval within each month over the period 1936–70, measured at 4 m of depth along the coast by coastal liners (Sætre 1973). Sea temperatures were used, selecting the station closest to the mouth of each river (Fig. 1). The distance between the station and the river mouth varied between 15 and 150 km. Anadromous brown trout usually stay in coastal waters within 100 km of the river mouth in Norway (Jensen 1968; Jonsson 1985; Berg and Berg 1987).

Statistical Analysis

Statistical relationships between variables were described using linear least-squares and binomial regression analysis.

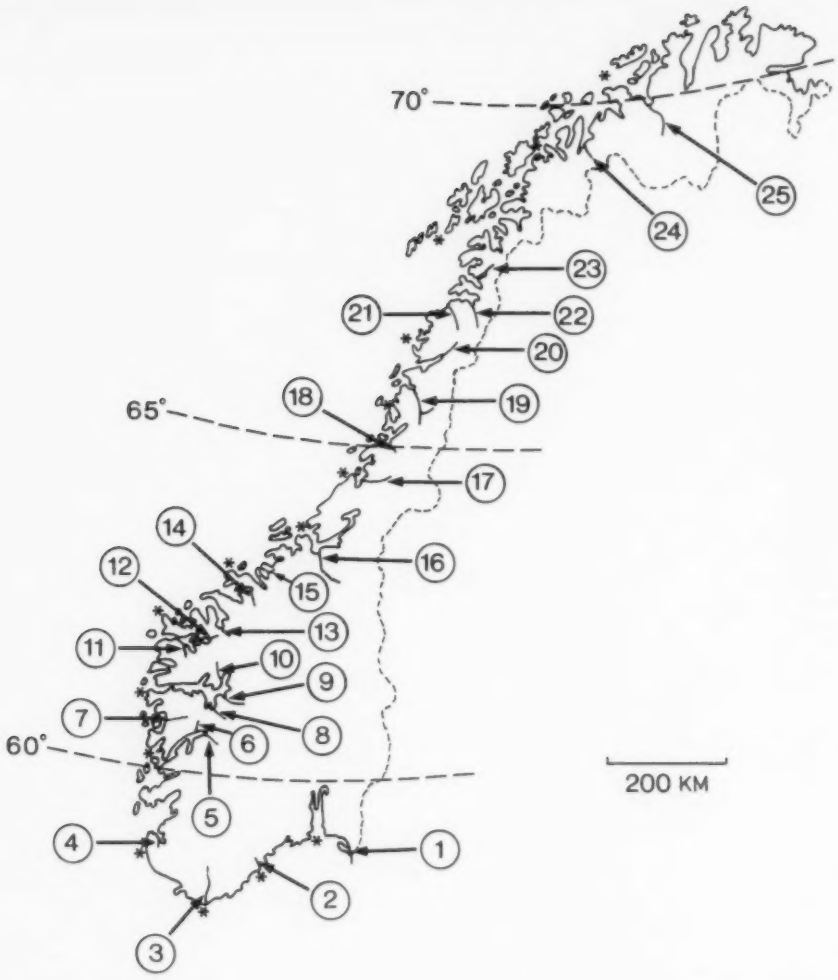

FIG. 1. Locations of 25 rivers in Norway where anadromous brown trout were studied. Names of the rivers are given in Table 1. Locations where sea temperature are measured are indicated with an asterisk.

When the assumption of normality was not met, we used the nonparametric Kendall's rank correlation analysis to test relationship between variables (Sokal and Rohlf 1981). The coefficient of determination (R^2) was used to describe the strength of the relationship. Multiple regression analysis was used to account for the influence of factors after smoltification (e.g. sea temperature and growth the first summer at sea) on the relationship between longevity and factors at the parr stage (e.g. river temperature and growth during the second year in freshwater.

Results

Longevity

Longevity of males and females increased significantly ($P < 0.05$, $R^2 = 0.54$ and 0.57 in males and females, respectively) with increasing latitude (Fig. 2A; Table 2). From 58 to 70°N, longevity increased by approximately 50%. The largest changes in longevity appeared between 58 and 60°N, and little elsewhere. Throughout the paper, one outlyer is observed in males, the River Aurlandselva (population 8). Omitting this river from the analysis the coefficient of determination of males and females increased to $R^2 = 0.79$ and 0.63, respectively. This river is cold relative to its latitude due to regulation for hydroelectric purposes. We are not aware of any other abiotic characteristic of this river which would cause divergent life history traits in the brown trout.

Moreover, longevity of both sexes decreased significantly ($P < 0.001$) with increasing sea and river temperatures (Fig. 2B, 2C; Table 2). In particular, longevity decreased among populations living at sea temperatures of more than 1800 degree-days. Eighteen hundred degree-days corresponds to approximately 60°N (Table 1). Brown trout feeding in the cold sea off northern Norway were approximately 50% older than those feeding in the warmer waters of the Skagerak. Furthermore, brown trout originating from cold rivers (glacier fed) were almost twice as old as those originating from warm (southern) rivers. In males, river temperature ($R^2 = 0.76$) explained a larger part of the variation in longevity than did sea

TABLE 1. Rivers sampled, latitude of river mouth, sampling periods, length increment (mm) during the second year in freshwater (river) and the first summer at sea, temperature (degree-days) during the growth season in freshwater and at sea, sample size, and longevity (yr) and body size (cm) of anadromous brown trout from 25 Norwegian rivers.

River	Latitude (°N)	Sampling period	Growth		Temperature		Sample size		Longevity		Body size	
			River	Sea	River	Sea	Male	Female	Male	Female	Male	Female
1. Enningdalselva	58.59	1983–86	74	204	2642	2164	46	46	4.7	5.5	35.5	40.0
2. Langangselva	58.30	1984	73	162	—	2048	—	36	—	5.0	33.1	37.3
3. Audna	58.05	1985–86	75	164	2382	1874	39	80	5.9	5.2	39.8	39.2
4. Imsa	58.53	1977–79	63	160	2873	1785	11	36	5.0	6.4	35.7	41.0
5. Eio	60.22	1982–85	48	162	1723	1858	34	27	—	8.2	38.0	36.9
6. Granvin	60.32	1982	56	118	—	1858	10	12	—	—	38.2	42.5
7. Vosso	60.38	1976–78	72	130	1822	1858	134	166	6.5	6.6	42.7	45.5
8. Aurlandselva	60.54	1985–87	38	110	1352	1717	31	47	11.0	10.4	70.6	57.7
9. Lærdalselva	61.07	1985	45	138	1480	1717	58	118	7.9	7.8	51.2	49.0
10. Jostedøla	61.23	1979–85	38	134	1055	1717	13	22	—	8.8	57.7	50.5
11. Ommedalselva	61.44	1985	45	113	1656	1647	57	90	7.5	11.1	42.8	44.6
12. Strynselva	61.55	1983–89	57	149	1818	1647	35	43	7.3	8.4	—	—
13. Korsbrekkelva	62.07	1986	53	133	—	1717	29	38	7.8	7.8	41.6	43.5
14. Eira	62.40	1987	47	94	1866	1717	27	58	7.8	8.6	43.4	40.8
15. Bævra	63.02	1986	51	144	—	1601	20	5	—	—	30.1	42.9
16. Gaula	63.20	1986	50	127	1249	1317	115	58	8.4	10.6	33.2	40.0
17. Namsen	64.28	1986	53	94	1556	1162	104	66	8.0	8.4	37.4	37.5
18. Åbjøra	65.04	1984	45	152	1585	1162	89	152	7.2	6.8	43.3	42.1
19. Vefsna	65.50	1982–85	45	112	1334	1093	31	44	—	9.5	41.0	43.3
20. Ranaelva	66.20	1979–86	44	119	892	1093	47	93	9.0	8.7	45.3	44.6
21. Beiarelva	67.02	1985–86	36	117	811	1080	64	80	8.3	8.3	43.3	44.0
22. Saltdalselva	67.07	1982–86	38	134	1149	1080	49	70	9.9	10.7	58.4	52.5
23. Kobbelv	67.37	1976–84	37	105	1129	1081	10	—	8.0	—	32.7	44.2
24. Skibotnelva	69.23	1981–85	41	148	1064	720	10	9	—	—	42.5	49.9
25. Alta	69.58	1984–85	42	114	1367	720	40	44	8.5	8.2	41.8	43.3

temperature ($R^2 = 0.50$) (Table 2). In contrast, for females, sea ($R^2 = 0.52$) and river ($R^2 = 0.49$) temperatures explained approximately the same amount of the variance in longevity (Table 2).

Longevity in males and females decreased significantly ($P < 0.001$) with increasing back-calculated length increment during the first summer at sea (Fig. 2E) and with back-calculated length increment during the second year in freshwater (Fig. 2F). With a doubling of the annual length increment in fresh and seawater, the longevity was reduced by about 40%. In both sexes, growth in freshwater explained a larger part of the variance of longevity ($R^2 = 0.68$ and 0.66 for males and females, respectively) than did growth at sea ($R^2 = 0.61$ and 0.49 for males and females, respectively) (Table 2). Furthermore, the linear least-squares regression of longevity on growth in freshwater was not significantly improved by including growth at sea in the analysis. Thus, conditions influencing the early stage of life, e.g. river temperature and corresponding growth, seem more important in determining the longevity of brown trout than conditions after smolting, e.g. sea temperature and corresponding growth.

Longevity of males and females increased significantly ($P < 0.05$) with body size given as length at sexual maturity (Fig. 2D). With an increase of 30 cm in body size, the longevity was increased by 40% in both sexes.

Body Size

We found no significant ($P > 0.05$) correlation between body size of either sex and latitude (Fig. 3A) or sea temperature (Fig. 3B). Moreover, we found no significant ($P > 0.05$) correlation between body size of males and river temperature (Fig. 3C). However, body size of females decreased significantly (Kendall's $\tau = -0.36$, $n = 20$, $P < 0.05$) with river temperature (Fig. 3C).

Furthermore, body size of both sexes was significantly (Kendall's $\tau = -0.43$ and -0.51, $n = 24$, $P < 0.01$ for males and females, respectively) correlated with back-calculated length increment during the second year in freshwater (Fig. 3E), but not with back-calculated length increment during the first summer at sea (Fig. 3D). With a doubling of the annual length increment in freshwater, body size was reduced by about 20%. As in the case of longevity, the conditions in the river (temperature with corresponding growth) were considerably more important for ultimate body size of both sexes than were temperatures and growth at sea.

Discussion

Longevity

Based on the present results, we reject the hypothesis that the longevity of brown trout is independent of latitude and temperature in freshwater and in the sea. Ambient water temperature, by influencing the metabolic rate, may be the single most important factor affecting the longevity of brown trout. The populations studied inhabit environments which differ considerably in temperature regimes. The southern stocks (58–63°N) experience seawater temperatures (measured as degree-days in the growth season) ranging from 1600 to 2100 degree-days, which are significantly higher than those experienced by their northern counterparts (68–70°N), which range from 600 to 800 degree-days. Higher freshwater temperatures are also recorded in the southern rivers compared with the northern rivers. The metabolic rate is probably slowed in the northern populations by the low temperatures.

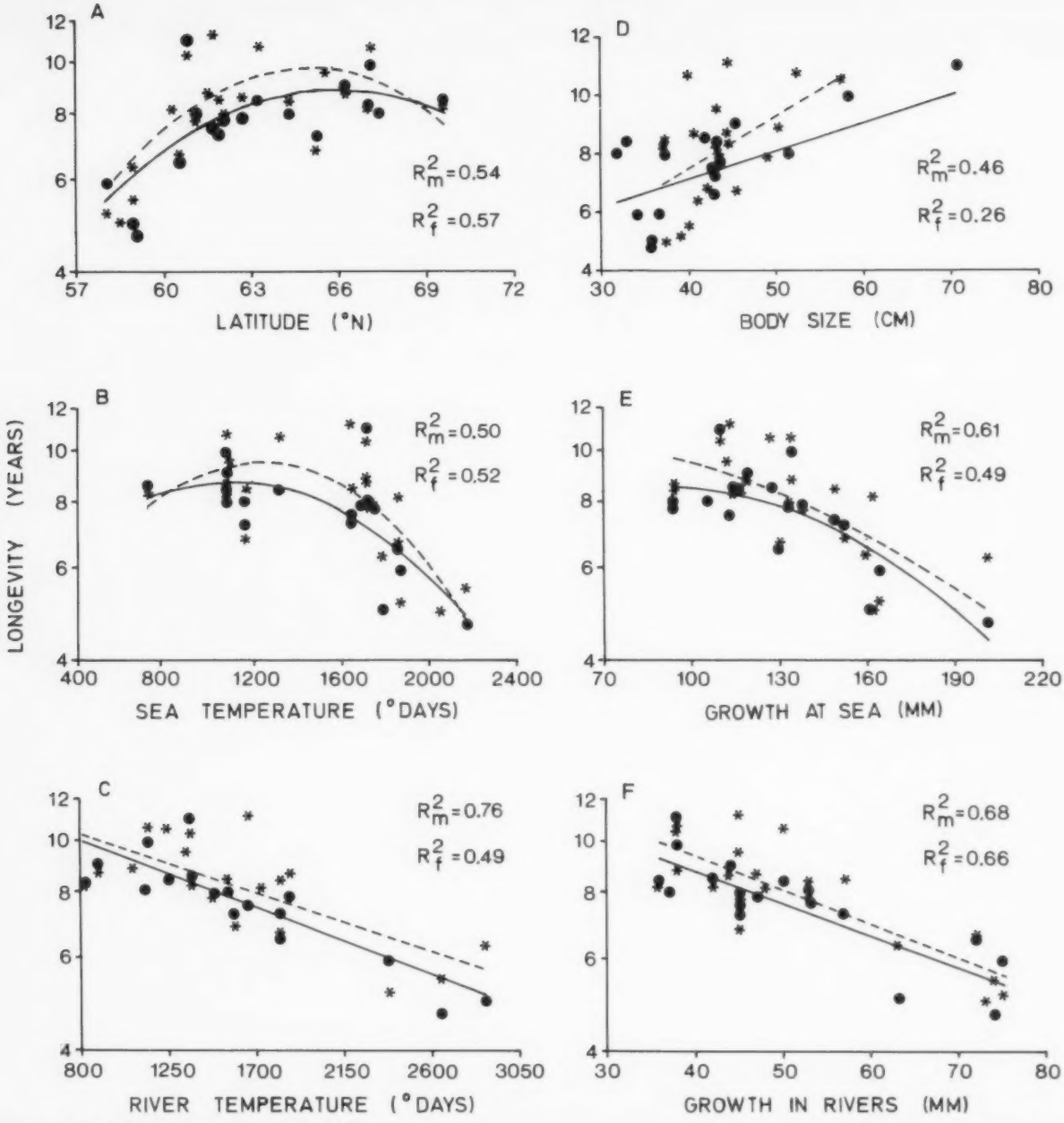

FIG. 2. Relationship between longevity and (A) latitude, (B) sea temperature within the growth season, (C) river temperature within the growth season, (D) body size, (E) length increment during the first summer at sea, and (F) length increment during the second year in freshwater. Males are indicated by solid circles and lines, and females are indicated by asterisks and broken lines. Coefficient of determination is given for males (R^2_m) and females (R^2_f). Regression statistics are given in Table 2.

A comparable decrease in longevity with latitude and water temperature may occur in other fish species too. For instance, Leggett and Carscadden (1978) observed semelparity and shorter life span among southern populations and iteroparity and longer life spans among northern populations of American shad on the east coast of North America. Glebe and Leggett (1981) attributed this life history difference at least partly to the warmer waters and higher metabolic rates in the southern part of the distribution area of American shad. Furthermore, within a species, one might expect longer life spans at high than at low altitudes because of the effects of water temperature. In parallel with this, some of the oldest brown trout are from high-altitude populations in cold-water lakes and rivers (Sømme 1941).

Longevity increased with decreasing growth rate. This agrees with what is found in other salmonid species (Jonsson et al.

TABLE 2. Regression statistics for the least-squares regression lines in Fig. 2 and 3 based on n measurements of longevity (Y, years) and six independent variables (X) yielded $Y = a + bX + cX^2$. R^2 = coefficient of determination. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Group	a	b	c	n	R^2
Log_{10} longevity vs. latitude (°N)					
Males	−13.761	0.446	-3.38×10^{-3}	18	0.54***
Females	−20.198	0.653	-5.03×10^{-3}	21	0.57***
Log_{10} longevity vs. sea temperature (degree-days)					
Males	0.600	5.84×10^{-4}	-2.52×10^{-7}	18	0.50***
Females	0.366	9.54×10^{-4}	-3.72×10^{-7}	21	0.52***
Log_{10} longevity vs. river temperature (degree-days)					
Males	1.112	−0.00014		17	0.76***
Females	1.104	−0.00012		19	0.48***
Log_{10} longevity vs. growth at sea (mm)					
Males	0.756	4.01×10^{-3}	-2.26×10^{-5}	18	0.61***
Females	1.012	8.71×10^{-4}	-1.22×10^{-5}	21	0.49***
Log_{10} longevity vs. growth in rivers (mm)					
Males	1.183	−0.00602		18	0.68***
Females	1.232	−0.00646		21	0.66***
Longevity vs. body size					
Males	2.801	0.1135		17	0.46**
Females	0.450	0.1757		20	0.26*

1984; Thorpe 1986). Within populations, fast growers usually mature younger and are more short-lived than slow growers. Although there is variation among stocks, this general trend also occurred among populations of anadromous brown trout. We hypothesize that this may be due to lower metabolic rate per unit weight during immature growth in slow- than in fast-growing fish so that each cell may last longer.

Longevity increased with body size. This may also be because the specific rate of metabolism decreases with increasing body size. This general relationship holds true irrespective of whether the animal is homeothermic or poikilothermic (Fenchel 1974; Pauly 1980; Peters 1983). Brown trout, however, became large because they continued to grow even at older ages, so their large body size was partly a consequence of age itself.

Body Size

We cannot reject the hypothesis that maximum body size of brown trout is independent of latitude and temperature in fresh or seawater. This disagrees with Bergmann's rule, which states that maximum body size increases with latitude and decreasing ambient temperature. Moreover, our results on body size are contrary to earlier results reported from related salmonids (Withler 1966; Pauly 1980) and from more distantly related poikilotherms (Ray 1960; Lindsey 1966). However, disagreements with Bergmann's rule are also found in body sizes of homeotherms such as otter (*Lutra canadensis*), mink (*Mustela vison*), jaguar (*Felis onca*), ocelot (*Felis pardalis*), fisher (*Martes pennanti*), mouse (*Microtus pennsylvanicus*), shrew (*Sorex cinereus*), and wolf (*Canis lupus*) (McNab 1971).

Because body size cannot be related to heat conservation in poikilotherms, the lack of relationship between body size and latitude may be expected. Maximum body sizes of fish are chiefly related to the rate of individual growth and age at first reproduction (Reznick 1983; Jensen 1985). Growth rate is largely determined by the quality, quantity, and availability of food (Boisclair and Leggett 1989a, 1989b) and abiotic factors such as temperature and oxygen content of the water (Elliott 1975; Jensen 1990). Fish have indeterminate and flexible growth, and the net energy gain from the food determines the annual size increment of the immatures (Weatherly and Gill 1987). At old age, however, growth slows among immatures (Pauly 1981), and even more so among adults due to energetic costs associated with reproduction (Williams 1966; Reznick and Endler 1982). Lien (1978) reported that brown trout in Øvre Heimdalsvatn, Norway, invested 46% of the total energy into annual reproduction. Furthermore, annual length increment of anadromous brown trout is reduced by about 50% or more in the year of first reproduction, relative to immatures of corresponding ages (Johnsson 1985, 1989).

Body size increased with decreasing temperature and growth rate in freshwater, but not with temperature and growth rate at sea. The size of the fish is at least partly a result of age at sexual maturity. Anadromous brown trout live in freshwater as parr and from autumn to spring as sea-run migrant fish. Sea-run migrants feed very little while staying in freshwater (Jonsson and Gravem 1985). We may therefore infer that it is either (1) slow developmental rates as parr in freshwater or (2) low metabolic rates of immature sea-run migrant fish in freshwater during winter that delay the process of maturation so they will grow larger. At present, we see no further evidence supporting the first hypothesis, although Ivanova and Volodin (1981) found that the incidence of sexual maturation in smelt (*Osmerus eperlanus*) varied in relation to physicogeographical features and water temperature during larval development. However, within populations of anadromous salmonids, there is a tendency towards younger, not older, sea ages at maturity among slowly developing parr (e.g. due to low temperature) (Isaksson 1983; Jonsson 1985). The alternative hypothesis, on the contrary, is at least partly supported by Rowe and Thorpe (1990) who stated that decisions about whether or not a fish will mature are taken during winter and early spring. They found that if spring growth of Atlantic salmon parr males was low, maturation was suppressed. Based on our observations, we hypothesize that age at maturity decreases with increasing metabolic rates during spring, and therefore, body size of anadromous brown trout increases with decreasing river temperature.

All in all, temperature influencing development and metabolic rates in freshwater appears to be more important than conditions in the sea for the observed variation in longevity and body size in anadromous brown trout.

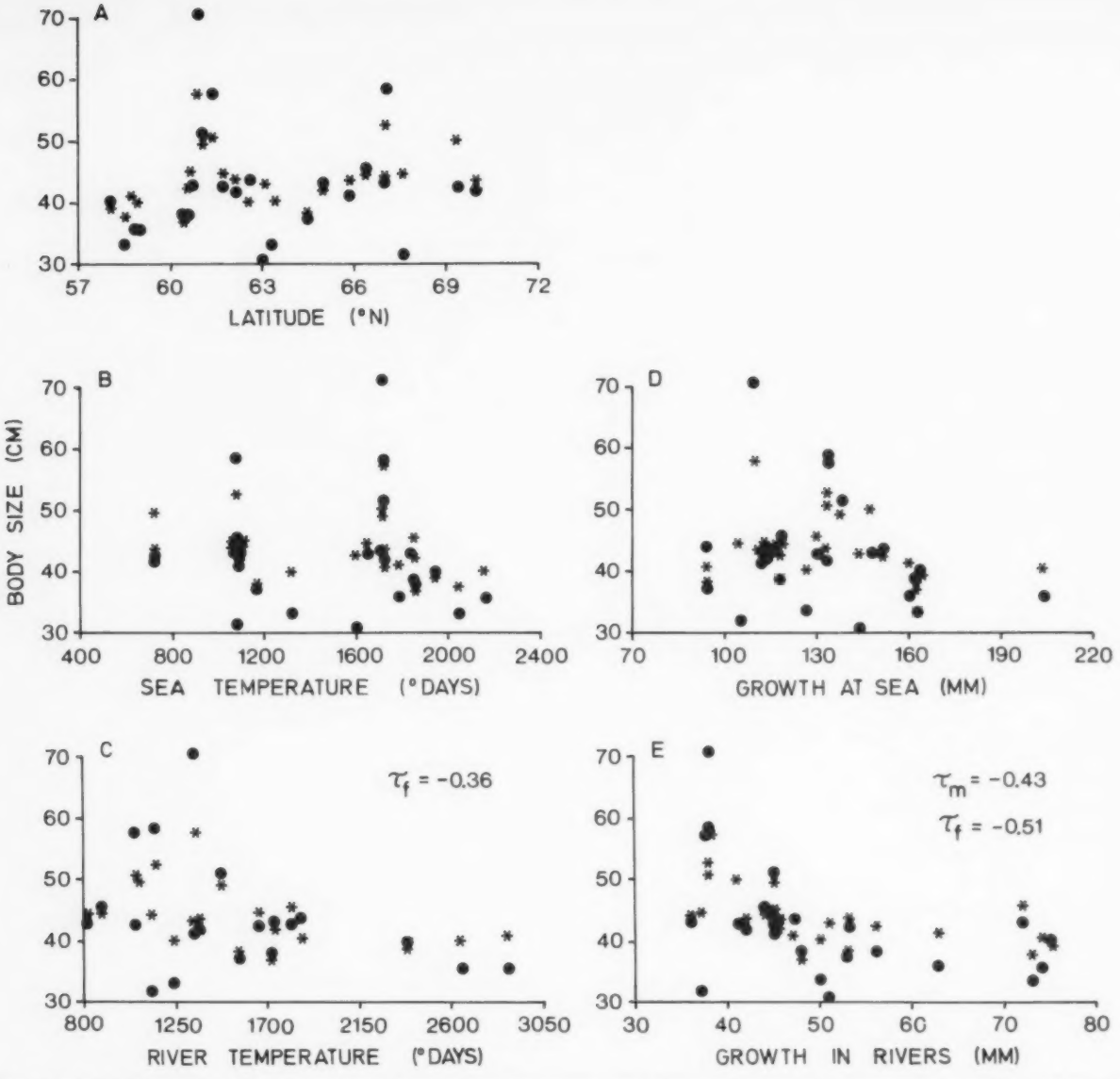

FIG. 3. Relationship between body size and (A) latitude, (B) sea temperature within the growth season, (C) river temperature within the growth season, (D) length increment during the first summer at sea, and (E) length increment during the second year in freshwater. Males are indicated by solid circles and lines, and females are indicated by asterisks and broken lines. Significant ($P < 0.05$) relationships between variables are given by their Kendall's rank correlation coefficient for males (τ_m) and females (τ_f).

References

ALM, G. 1959. Connection between maturity, size, and age in fishes. Inst. Freshwater Res. Drottningholm Rep. 40: 5–145.

BEAMISH, F. W. H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. II. Influence of weight and temperature on respiration of several species. Can. J. Zool. 42: 177–188.

BERG, O. K., AND M. BERG. 1987. Migrations of sea trout, *Salmo trutta* L., from the Vardnes river in northern Norway. J. Fish Biol. 31: 113–121.

BERG, O. K., AND B. JONSSON. 1990. Growth and survival rates of the anadromous brown trout, *Salmo trutta*, from the Vardnes River, northern Norway. Environ. Biol. Fishes 29: 145–154.

BOISCLAIR, D., AND W. C. LEGGETT. 1989a. Among-population variability of fish growth: I. Influence of the quantity of food consumed. Can. J. Fish. Aquat. Sci. 46: 457–467.

1989b. Among-population variability of fish growth: II. Influence of prey type. Can. J. Fish. Aquat. Sci. 46: 468–482.

BONNER, J. T. 1965. Size and cycles. An essay on the structure of biology. Princeton University Press, Princeton, NJ. 219 p.

BORGSTRØM, R., AND J. HEGGENES. 1989. Smoltification of sea trout (*Salmo trutta*) at short length as an adaptation to extremely low summer stream flow. Pol. Arch. Hydrobiol. 35: 375–384.

BRETT, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21: 1183–1226.

BRETT, J. R., AND T. D. D. GROVES. 1979. Physiological energetics, p. 279–352. *In* W. S. Hoar, D. J. Randall, and J. R. Brett [ed.] Fish physiology. Vol. VIII. Bioenergetics and growth. Academic Press, New York, NY.

CALDER, W. A. III. 1984. Size, function, and life history. Harvard University Press, Cambridge, 431 p.

CARLANDER, K. D. 1980. Caution on the use of the regression method of back-calculating lengths from scale measurements. Fisheries 6: 2–4.

DAHL, K. 1910. Alder og vekst hos laks og ørret belyst ved studier av deres skjæl. Landbruksdepartementet, Kristiania. 115 p.

1917. Studier og forsøk over ørret og ørretvand. Centraltrykkeriet, Kristiania. 107 p.

DELLEFORS, C., AND U. FAREMO. 1988. Early sexual maturation in males of wild sea trout, *Salmo trutta* L., inhibits smoltification. J. Fish Biol. 33: 741–749.

ELLIOTT, J. M. 1975. The growth rate of brown trout, *Salmo trutta* L., fed on maximum rations. J. Anim. Ecol. 44: 805–821.

FENCHEL, T. 1974. Intrinsic rate of natural increase: the relationship with body size. Oecologia 14: 317–326.

FROST, W. E., AND M. E. BROWN. 1967. The trout. Collins, London. 286 p.

GLEBE, B. D., AND W. C. LEGGETT. 1981. Latitudinal differences in energy allocation and use during the freshwater migrations of American shad (*Alosa sapidissima*) and their life history consequences. Can. J. Fish. Aquat. Sci. 38: 806–820.

ISAKSSON, A. 1983. Sea ranching of salmon in Iceland — present status, p. 23–42. *In* C. Eriksson, M. P. Farranti, and P. O. Larsson [ed.] Cost 46/4 Workshop sea ranching of Atlantic salmon. Proceedings. Commission of the European Communites, Älvkarleby, Sweden.

IVANOVA, M. N., AND V. M. VOLODIN. 1981. Variability in rate of sexual maturation in freshwater populations of the smelt (*Osmerus eperlanus*). J. Ichthyol. 21: 28–37.

JENSEN, A. J. 1990. Growth of young migratory brown trout *Salmo trutta* correlated with water temperature in Norwegian rivers. J. Anim. Ecol. 59: 603–614.

JENSEN, A. L. 1985. Relations among net reproductive rate and life history parameters for lake whitefish (*Coregonus clupeaformis*). Can. J. Fish. Aquat. Sci. 42: 164–168.

JENSEN, K. W. 1968. Seatrout (*Salmo trutta*, L.) of the River Istra, Western Norway. Inst. Freshwater Res. Drottningholm Rep. 48: 187–213.

JONSSON, B. 1976. Comparison of scales and otoliths for age determination in brown trout, *Salmo trutta* L. Norw. J. Zool. 24: 295–301.

1977. Demographic strategy in a brown trout population in Western Norway. Zool. Scr. 6: 255–263.

1985. Life history patterns of freshwater resident and sea-run migrant brown trout in Norway. Trans. Am. Fish. Soc. 114: 182–194.

1989. Life history and habitat use of Norwegian brown trout (*Salmo trutta*). Freshwater Biol. 21: 71–86.

JONSSON, B., AND F. GRAVEM. 1985. Use of space and food by resident and migrant brown trout, *Salmo trutta*. Environ. Biol. Fishes 14: 281–293.

JONSSON, B., K. HINDAR, AND T. G. NORTHCOTE. 1984. Optimal age at sexual maturity of sympatric and experimentally allopatric cutthroat trout and Dolly Varden charr. Oecologia 61: 319–325.

L'ABÉE-LUND, J. H., A. J. JENSEN, AND B. O. JOHNSEN. 1990. Interpopulation variation in male parr maturation of anadromous brown trout (*Salmo trutta*) in Norway. Can. J. Zool. 68: 1983–1987.

L'ABÉE-LUND, J. H., B. JONSSON, A. J. JENSEN, L. M. SÆTTEM, T. G. HEGGBERGET, B. O. JOHNSEN, AND T. F. NÆSJE. 1989. Latitudinal variation in life-history characteristics of sea-run migrant brown trout *Salmo trutta*. J. Anim. Ecol. 58: 525–542.

LEA, E. 1910. On the methods used in herring investigations. Publ. Circ. Cons. Perm. Int. Explor. Mer 53: 7–25.

LEGGETT, W. C., AND J. E. CARSCADDEN. 1978. Latitudinal variation in reproductive characteristics of American shad (*Alosa sapidissima*): evidence for population specific life history strategies in fish. J. Fish. Res. Board Can. 35: 1469–1478.

LIEN, L. 1978. The energy budget of the brown trout population of Øvre Heimdalsvatn. Holarct. Ecol. 1: 279–300.

LINDSEY, C. C. 1966. Body size of poikilotherm vertebrates at different latitudes. Evolution 20: 456–465.

MAYR, E. 1956. Geographical character gradients and climatic adaption. Evolution 10: 105–108.

MCNAB, B. K. 1971. On the ecological significance of Bergmann's rule. Ecology 52: 845–854.

NIEMELÄ, E., AND R. L. MCCOMAS. 1985. Preliminary seatrout (*Salmo trutta* L.) investigations in the Teno River. Int. Counc. Explor. Sea C. M. 1985/M:24: 11 p.

NIKOLSKY, G. V. 1963. The ecology of fishes. Academic Press, London. 352 p.

NORDENG, H. 1977. A pheromone hypothesis for homeward migration in anadromous salmonids. Oikos 28: 155–159.

PAULY, D. 1980. On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. J. Cons. Int. Explor. Mer 39: 175–192.

1981. The relationship between gill surface area and growth performance in fish: a generalization of von Bertalanffy's theory of growth. Meeresforschung 28: 251–282.

PETERS, R. H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge. 329 p.

PRATTEN, D. J., AND W. M. SHEARER. 1983. The migrations of North Esk sea trout. Fish. Manage. 14: 99–113.

RAY, C. 1960. The application of Bergmann's and Allen's rule to the poikilotherms. J. Morphol. 106: 85–108.

REZNICK, D. 1983. The structure of guppy life histories: the tradeoff between growth and reproduction. Ecology 64: 862–873.

REZNIK, D., AND J. A. ENDLER. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36: 160–177.

RICKER, W. E. 1979. Growth rates and models, p. 677–743. *In* W. S. Hoar, D. J. Randall, and J. R. Brett [ed.] Fish physiology. Vol. VIII. Bioenergetics and growth. Academic Press, New York, NY.

ROSSELAND, L. 1965. Rapport om utførte lakseundersøkelser m.v. Vedlegg til Fiskeriinspektørens årsmelding for årene 1951–1962. Landbruksdepartementet, Oslo.

ROWE, D. K., AND J. E. THORPE. 1990. Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reduction in feeding and growth during spring months. Aquaculture 86: 291–313.

SÆTRE, R. 1973. Temperature and salinity normals for the surface layer in Norwegian coastal waters. Fiskets Gang 59: 166–172.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. W. H. Freeman and Company, San Francisco, CA. 859 p.

SØMME, I. D. 1941. Ørretboka. Jacob Dybwads Forlag, Oslo. 591 p.

THORPE, J. E. 1986. Age at first maturity in Atlantic salmon, *Salmo salar*: freshwater period influences and conflicts with smolting, p. 7–14. *In* D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

WEATHERLY, A. H., AND H. S. GILL. 1987. The biology of fish growth. Academic Press, London. 443 p.

WILLIAMS, G. C. 1966. Natural selection, the cost of reproduction, and a refinement of Lack's principle. Am. Nat. 100: 687–690.

WITHLER, I. L. 1966. Variability in life history characteristics of steelhead trout (*Salmo gairdneri*) along the Pacific coast of North America. J. Fish. Res. Board Can. 23: 365–393.

WOOTTON, R. J. 1990. Ecology of teleost fishes. Chapman and Hall, London. 404 p.

Crustacean Zooplankton Food Web Structure in Lakes of Varying Acidity

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Havens, K. E. 1991. Crustacean zooplankton food web structure in lakes of varying acidity. Can. J. Fish. Aquat. Sci. 48: 1846–1852.

Crustacean zooplankton food web models were constructed for 25 softwater United States lakes of varying pH (4.7–7.2). Eight lakes on the North Mountain Plateau of Pennsylvania were sampled in 1987, and data from 17 Adirondack Mountain, NY, and White Mountain, NH, lakes were taken from Confer et al. (1983. Can. J. Fish. Aquat. Sci. 40: 36–42). In the most acidic lakes, the zooplankton food webs were simplified, with only two or three crustacean species; the highest pH lakes contained 6–10 species. Positive relationships were found between lake water pH and species richness, total number of predator–prey interactions, average number of interactions per species, and average generalization of predators. Inverse relationships were found between pH and both the degree of omnivory and cannibalism in the webs, perhaps reflecting the adaptiveness of those feeding strategies under circumstances where energy flow from adjacent trophic levels is reduced. Reduced energy flow may limit the number of trophic levels in the most acidic lakes. Lakes of pH < 5 had only two or three modal trophic levels, while lakes of pH > 5 had three, four, or five levels.

On a élaboré des modèles de pyramide alimentaire fondés sur un zooplancton de crustacés pour 25 lacs d'eau douce des États-Unis dont le pH variait entre 4,7 et 7,2. Huit lacs situés sur le North Mountain Plateau, Pennsylvanie, ont été échantillonnés en 1987; les données provenant de 17 lacs des Adirondack, NY, et des White Mountains, NH, sont tirées de Confer *et al.* (1983. J. can. sci. halieut. aquat. 40 : 36–42). Dans les lacs les plus acides, les pyramides alimentaires zooplanctoniques étaient simplifiées, elles ne comptaient que deux à trois espèces de crustacés; les lacs au pH le plus élevé contenaient 6 à 10 espèces. Des rapports positifs ont été établis entre le pH de l'eau des lacs et la richesse spécifique, le nombre total d'interactions entre les prédateurs et les proies, le nombre moyen d'interactions par espèce ainsi que la généralisation moyenne des prédateurs. Des relations inverses ont été déterminées entre le pH et le degré de polyphagie ainsi que de cannibalisme dans les pyramides alimentaires; cela témoigne peut-être de la souplesse des stratégies d'alimentation dans des conditions où la circulation d'énergie à partir de niveaux trophiques contigus est réduite. La circulation réduite de l'énergie peut limiter le nombre de niveaux trophiques dans les lacs les plus acides. Les lacs dont le pH est inférieur à 5 n'avaient que deux à trois niveaux trophiques modaux alors que les lacs dont le pH était supérieur à 5 comptaient trois, quatre ou cinq.

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Six decades have passed since Klugh and Miller (1926) first studied the effects of lowered pH on *Daphnia magna* and Lowndes (1926) examined the distribution of *Cyclops* spp. in Ontario lakes of varying acidity. In the years that have followed, considerable research has been done to assess the effects of acidification on freshwater zooplankton. Multilake surveys (e.g. Sprules 1975; Roff and Kwiatkowski 1977; Yan and Strus 1980; Confer et al. 1983; Keller and Pitblado 1984; Carter et al. 1986; MacIsaac et al. 1987; Pinel-Alloul et al. 1990), whole-lake acidification experiments (e.g. Schindler et al. 1985; Watras and Frost 1989), and mesocosm studies (e.g. Havens and DeCosta 1987; Havens and Heath 1989) have demonstrated several consistent effects. Zooplankton biomass and species richness decline with increasing acidity. Daphnids are eliminated, while smaller crustaceans (especially *Bosmina longirostris* and *Diaptomus minutus*) and rotifers become the dominant herbivores. With the loss of fish populations that also occurs (Mills et al. 1987), invertebrates like corixid beetles, *Chaoborus* larvae, and cyclopoids become the top predators in the limnetic zone (Eriksson et al. 1980; Henrikson and Oscarson 1981).

All zooplankton species are members of food webs. Therefore, chemical stressors like acidification have both direct toxic effects and indirect effects due to altered biotic interactions in the food web. Yet, only a few workers have examined the effects of acidification on interspecific competition (Janicki and DeCosta 1984; Havens and DeCosta 1985a) and predation (Eriksson et al. 1980; Havens and DeCosta 1985b); the effects on overall food web structure and function have not been quantified. Although Sprules and Bowerman (1988) constructed food webs for over 500 North American lakes, including acid and nonacid subsets, they did not investigate the relationships between food web parameters and lake water acidity. Lane and Blouin (1985) used loop analysis to investigate food web structure in three acid-impacted Nova Scotia lakes, but no lakes of pH above 5.4 were studied.

In the present study, food web structure was compared in 25 softwater oligotrophic lakes of varying pH (4.7–7.2) in order to obtain a better understanding of zooplankton community responses to lake acidification.

Materials and Methods

Zooplankton Data Set

Zooplankton data (presence or absence of 29 crustacean species) were obtained from 25 moderate-altitude lakes in the acid-sensitive region of the northeastern United States. Data from 10 Adirondack Mountain, New York, lakes (location 73–74°W longitude, 43–44°N latitude, elevation 614–872 m) and seven White Mountain, New Hampshire, lakes (location 71–72°W longitude, 43–44°N latitude, elevation 212–617 m) were taken from Confer et al. (1983). Three humic lakes (Lake Constance, Black Pond, and Upper Hall Lake) were excluded because studies have shown that the humic lake zooplankton differs from that found in clearwater lakes of similar pH (Overrein 1980; Havens 1989). Zooplankton data from eight clearwater lakes on the North Mountain Plateau (location 75–76°W longitude, 41°N latitude, elevation 630–764 m) of northeastern Pennsylvania were also included in the data set. I sampled those lakes in summer 1987. To be consistent with Confer et al. (1983), only species accounting for >0.1% of numerical abundance were included. The complete data set consists of lakes which are as nearly alike as possible except for differences in acidity. All are headwater lakes which are thermally stratified in midsummer and are soft water and oligotrophic. Furthermore, since the lakes are remote, none is influenced by nearby sources of heavy metal pollution.

Zooplankton Collection and Enumeration

Confer et al. (1983) collected zooplankton once in July and once in August 1979 at three limnetic stations in the Adirondack and White Mountain lakes. A 3-L transparent van Dorn bottle was used to take water from three to five depths, including 0.5 m, just above the metalimnion, and within the metalimnion. They pooled concentrated samples for enumeration and determined pH using a laboratory meter and water samples taken at 0.5 m depth.

For the North Mountain lakes, I used sampling methods which were similar to those of Confer et al. (1983). I sampled once in June and once in July 1987. At each lake, samples were taken at 1-m intervals from the surface to the bottom with a 3-L van Dorn bottle. Three stations were sampled and the concentrated samples were pooled for counting. This was done by pouring collected water through a 58-μm net, backflushing the retained animals into a Whirl-pak bag, and preserving them in 4% formalin–sucrose (Prepas 1978). The sampling method used herein and by Confer et al. (1983) has the advantage of giving a more constant efficiency than is possible with a plankton net. With the latter technique, net clogging occurs when large algae are dominant. Since larger cells increase in importance with acidification (Schindler 1990; Havens and Heath 1991), sampling with a net would bias the data because efficiency would be lower in the more acidic lakes. Zooplankton samples from the North Mountain lakes were enumerated at 20× magnification using a dissecting microscope. Aliquots were examined until at least 200 crustaceans had been counted. Rotifers were not enumerated because the concentration procedure did not effectively retain common nonloricate species like *Polyarthra* and because the Confer et al. (1983) data set included only crustaceans. Species densities were determined assuming 100% sampling efficiency. Surface pH was determined in situ using a Sargent-Welch portable meter. Reported values are based on mean hydrogen ion concentrations determined for the two samplings at each lake. The June to July variation in pH for the North Mountain Lakes was minimal, ranging from 0 to 0.3 unit.

Food Web Analyses

Pictorial food web models were constructed using the presence/absence data for zooplankton species in the three regions. For the North Mountain lakes, these data are presented in Tables 1 and 2; for the Adirondack and White Mountain lakes, see Confer et al. (1983). Because Confer et al. (1983) combined data from two sampling dates to generate their presence/absence table, I used the same approach herein to maintain consistency within the data set. This approach added a single species each to the data for Wild Fowl Lake (pH 5.5) and Lake Jean (pH 5.6). Information on the prey of each zooplankton species was obtained from various sources (e.g. Gliwicz 1977; Brandl and Fernando 1978; Lane 1978; Kerfoot 1980; Nero and Sprules 1986), especially Sprules and Bowerman (1988) who presented a "look up" table of prey for North American crustacean zooplankton. I assumed that the diet of each predator did not vary from lake to lake. Nero and Sprules (1986) showed that for one zooplankton predator (*Mysis*), little lake to lake variation in prey preference occurred. No other studies have been done to investigate genetic variation in prey preference of predatory zooplankton. To determine exact predator–prey relationships, it is necessary to perform gut content analyses of all predator species. Since this was not done in the Confer et al. (1983) study, such a prerequisite for constructing food webs would have precluded the use of their data. Furthermore, the absence of particular prey type in a predator's gut does not always represent lack of an interaction. Rather, it might simply indicate that a prey type was not consumed during a period prior to sampling equal to the predator's gut passage time.

Each food web model was analyzed visually and seven parameters of food web structure were determined following Pimm (1982), Cohen (1978), Schoener (1989), and Sprules and Bowerman (1988):

(1) Species richness — Total number of species, exclusive of phytoplankton.

(2) Number of interactions — Total number of predator-prey interactions, not including cannibalism, and counting each cycle (species A preys on B and vice versa) as a single interaction.

(3) Number of interactions per species — Total number of interactions divided by the species richness.

(4) Generalization — Average number of zooplankton prey eaten per predator in the food web.

(5) Degree of cannibalism — Number of cannibalistic species divided by the total number of species in the web.

(6) Degree of omnivory — Number of omnivorous species divided by the total number of species in the web.

(7) Modal number of trophic levels (Cohen's maximum chain length) — All possible pathways except cannibalism were traced from the base of the food web (the phytoplankton) to each top predator. The length of each path was recorded as the number of links plus one. The modal length of the paths is the modal number of trophic levels. In accordance with Cohen (1978), the longest modal path length was chosen in the case of a tie.

Regression analyses were performed using SAS statistics version 5 (SAS Institute 1985) and the Kent State University IBM mainframe computer. Correlation coefficients reported herein are Pearson's product-moment coefficients.

TABLE 1. Crustacean zooplankton species accounting for greater than 0.1% of zooplankton abundance in eight lakes of varying pH on the North Mountain Plateau, Pennsylvania, USA. Zooplankton taxa codes are defined in Table 2.

Lake	pH	CHAO	MEDA	MCYC	DMIN	BLON	CSPH	ALON	OPHR	PPED
Splashdam	4.7		×						×	
Ice House	4.8		×		×					
Redrock	5.0		×		×					×
Spring	5.2		×		×	×				
Shumans	5.3		×		×		×			
Wild Fowl	5.5		×		×	×				×
Jean	5.6		×		×	×	×			×
Rouse	6.1	×		×	×	×	×	×		

TABLE 2. Identification of zooplankton taxa codes used in Table 1 and Fig. 1.

Symbol	Species
ALON	*Alona* sp.
AQUA	*Alona quadrangularis*
BLON	*Bosmina longirostris*
CHAO	*Chaoborus* sp.
CSCU	*Cyclops scutifer*
CSPH	*Chydorus sphaericus*
DCAT	*Daphnia catawba*
DDUB	*D. dubia*
DLEU	*Diaphanosoma leuchtenbergianum*
DMIN	*Diaptomus minutus*
DSAN	*D. sanguineus*
DSPA	*D. spatulocrenatus*
ELAC	*Epischura lacustris*
HGIB	*Holopedium gibberum*
MCYC	*Microcyclops* sp.
MEDA	*Mesocyclops edax*
OPHR	*Ophryoxus* sp.
PPED	*Polyphemus pediculus*
SCRY	*Sida crystallina*
TRPA	*Tropocyclops prasinus*

Results and Discussion

Food webs of the most acidic lakes were greatly simplified, with fewer species and fewer interactions than in the higher pH lakes (Fig. 1). This simplification of food web structure is consistent with Odum's (1985) prediction of community response to stress and reflects the loss of all but the most acid-tolerant species in the food webs of the lowest pH lakes. When all 25 lakes were considered, there was a positive relationship between food web complexity and pH; both species richness and the number of interactions in the food web increased with increasing pH (Fig. 2a, 2b). The correlations between species richness and pH ($r^2 = 0.90$, $p < 0.01$) and between the number of interactions and pH ($r^2 = 0.76$, $p < 0.01$) were highly significant.

The relationship between species richness and pH for the North Mountain subset was consistent with that reported by Confer et al. (1983) for the Adirondack and White Mountain subsets, i.e. approximately linear over the entire range of pH values. As Confer et al. (1983) noted, this is in contrast with the results of Sprules (1975), who found that species richness did not decline in Ontario lakes until pH was below 5.2, and Roff and Kwiatkowski (1977), who found that zooplankton diversity did not decline until pH was below 5.5. It is unclear whether these results indicate regional differences in zooplankton community response to acidification or differences in sampling methodology. The United States lakes described herein tend to be smaller, located at higher elevations, and more remote from heavy-metal sources than the Ontario lakes. All of these factors might affect zooplankton community structure. However, Sprules (1975) and Roff and Kwiatkowski (1977) also used large-diameter plankton nets (30 and 25 cm in diameter, respectively) which sampled a greater volume of water than the van Dorn bottle which I used at the North Mountain lakes and which Confer et al. (1983) used at the Adirondack and White Mountain lakes. As previously mentioned, the bottle sampling procedure also avoids the problem of plankton net clogging. Hence, two potentially important variations in sampling efficiency exist among the studies. Further research should be done using identical sampling techniques, so that we can determine whether significant regional differences exist in zooplankton response to acidification.

There was a significant positive relationship ($r^2 = 0.42$, $p < 0.05$) between the number of interactions per species and pH (Fig. 2c), with roughly 1.5 interactions per species in the most acid lakes and greater than 2 in the least acid lakes. These results provide support for the hypothesis that acidification decreases food web complexity. The observed relationship reflects the fact that in the most acid lakes, a relatively larger portion of prey was unavailable to the predators. For example, it was common to find only two or three herbivores in the most acid lakes, one of which was not effectively preyed upon by predatory zooplankton. The results also falsify the hypothesis that the number of interactions per species (linkage density) is constant among food webs of varying richness (Cohen and Briand 1984). The present study is among the first to show a significant change in linkage density among a set of comparably constructed food web models.

Generalization (Fig. 3a) also showed a positive relationship to lake water pH. The correlation between generalization and pH was highly significant ($r^2 = 0.68$, $p < 0.01$). In part, this might reflect a previously observed (Schoener 1989) relationship between generalization and species richness. Schoener examined 17 lentic food webs and found that generalization increased roughly twofold when richness increased from 3 to 30 species. The increase observed herein was much more pronounced; generalization increased fivefold when richness increased from 2 to 10 species. This suggests that acidification does reduce the average number of prey types available to a given predator in the web and that the results are not simply an artifact of the increased richness. It is noteworthy that Odum (1985) predicted a decline in redundancy (multiple pathways from producers to higher trophic levels) in food webs in response to stress and that this trend has been documented in

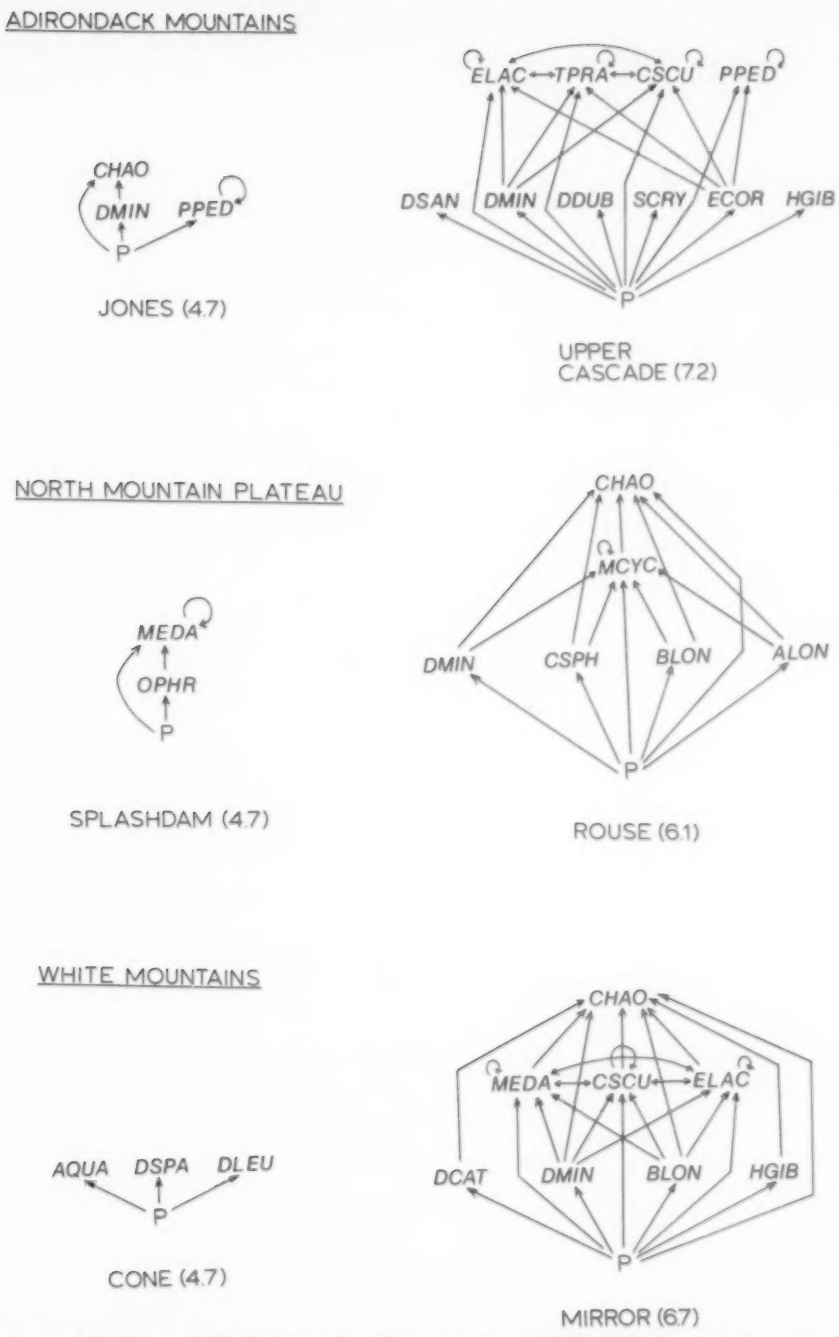

FIG. 1. Crustacean zooplankton food webs in the lowest and highest pH lakes of the three regions (pH values in parentheses). Single-headed arrows indicate predator-prey interactions, loops indicate cannibalism, and double-headed arrows indicate cycles. P represents the phytoplankton; see Table 2 for zooplankton taxa codes.

response to anthropogenic acidification of Ontario lakes (Schindler 1990).

Both the degree of cannibalism (Fig. 3b) and omnivory (Fig. 3c) were negatively related to lake water pH over the range from 4.7 to 6.5. Cannibalistic and omnivorous feeding modes may increase the fitness of animals faced with both a low number of prey choices at the adjacent trophic level and increased relative abundance of inedible prey. As previously mentioned, herbivores not effectively exploited by invertebrate predators were of greater importance in the acid lakes. Con-

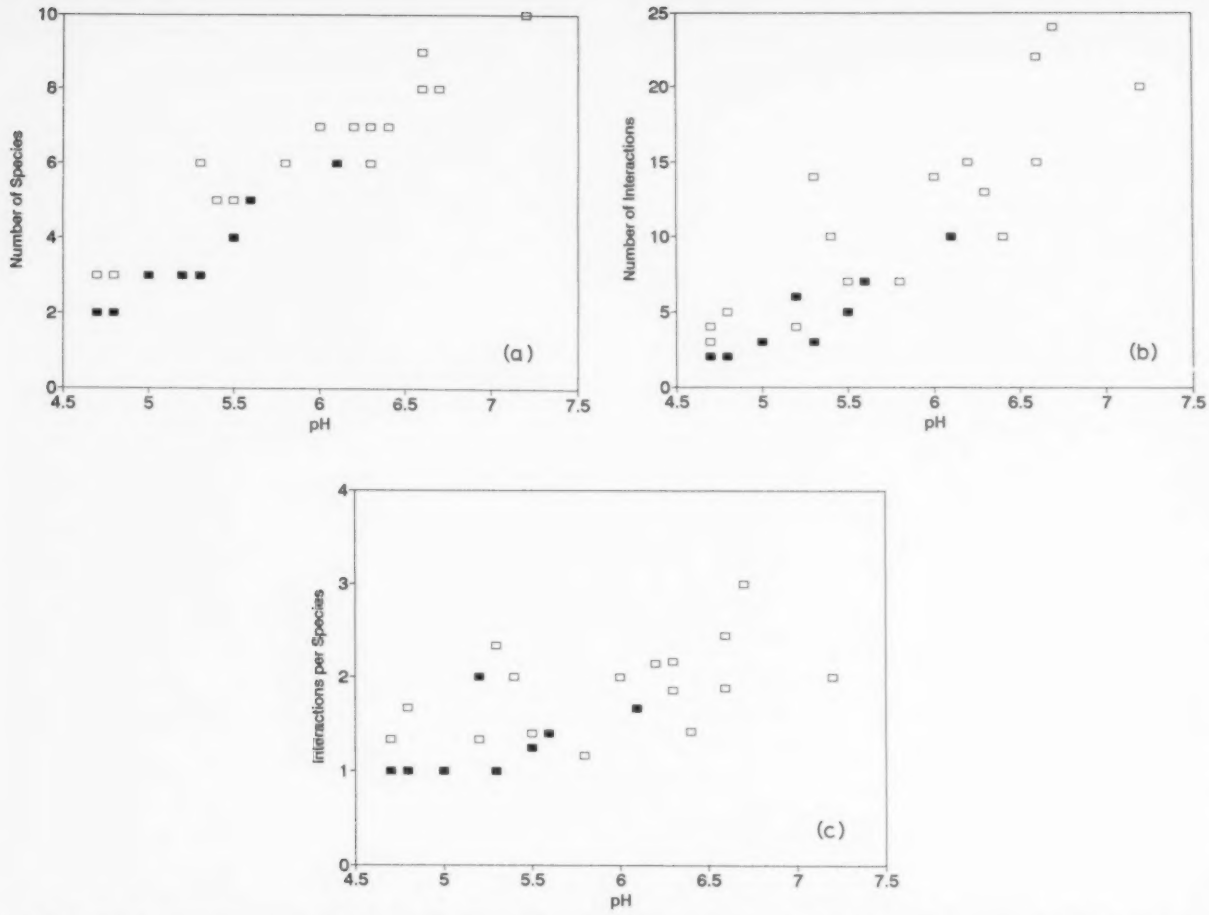

FIG. 2. Food web parameters versus pH. (a) Species richness; (b) number of interactions; (c) number of interactions per species. Black markers indicate the North Mountain lakes; open markers indicate the Adirondack and White Mountain lakes.

sequently, a single prey type (e.g. *Diaptomus minutus*) was sometimes available to a predator. When prey are scarce, cannibalism and/or omnivory might allow for persistence of predator populations which would otherwise become extinct.

In the highest pH lakes (>6.5), both the degree of cannibalism and omnivory increased, relative to values determined for the pH 6–6.5 lakes. This situation likely reflects a response to reduced energy flow through the food webs, similar to that seen in the most acid lakes. In the high-pH lakes, herbivores which are resistant to invertebrate predation were dominant (in terms of their relative richness). For example, in Upper Cascade Lake (pH 7.2), four of the six herbivore species were not available to the predators (Fig. 1). Under those circumstances, alternate feeding modes (cannibalism and omnivory) might allow invertebrate predator populations to survive periods when the few edible prey species are rare.

It has been suggested that cannibalism and omnivory add stability to a food web. Lane (1978) noted that "short negative feedback pathways like cannibalism can balance de-stabilizing effects of longer ones," and Pimm and Lawton (1977) showed that the model food webs, the addition of an omnivorous link increased the return time to equilibrium following perturbation (i.e. increased stability). The relationship between the degree of omnivory and food web stability is, however, quite controversial. Pimm and Lawton (1978) showed that increasing the rank of omnivory decreased the portion of model webs that were locally stable, but also increased the portion of stable webs having long return times. Paine (1988) cautioned that model webs might not always reflect biologically realistic ones, such that the application of the model web results to real systems might be limited. Therefore, while the greater incidence of omnivory in the acid lakes is intuitive based on energetic considerations, further research needs to be done before a cause and effect relationship can be stated.

With the exception of Cone Lake, NH (pH 4.7), which had only two zooplankton trophic levels, the modal number of trophic levels was three in all lakes with pH below 6.0 (Fig. 3d). In the less acid lakes, 50% had three, 37% had four, and 13% (a single lake, Mirror Lake, NH) had five modal trophic levels. Because inedible producers become more important with declining pH (Yan and Strus 1980; Schindler et al. 1985; Stokes and Yung 1986), it is possible that energetic constraints limit the number of trophic levels in the most acidic lakes. Lindeman (1942) first discussed the decline in energy flow to successively higher trophic levels, and Hutchinson (1959) noted that ultimately the amount of energy is insufficient to support an addi-

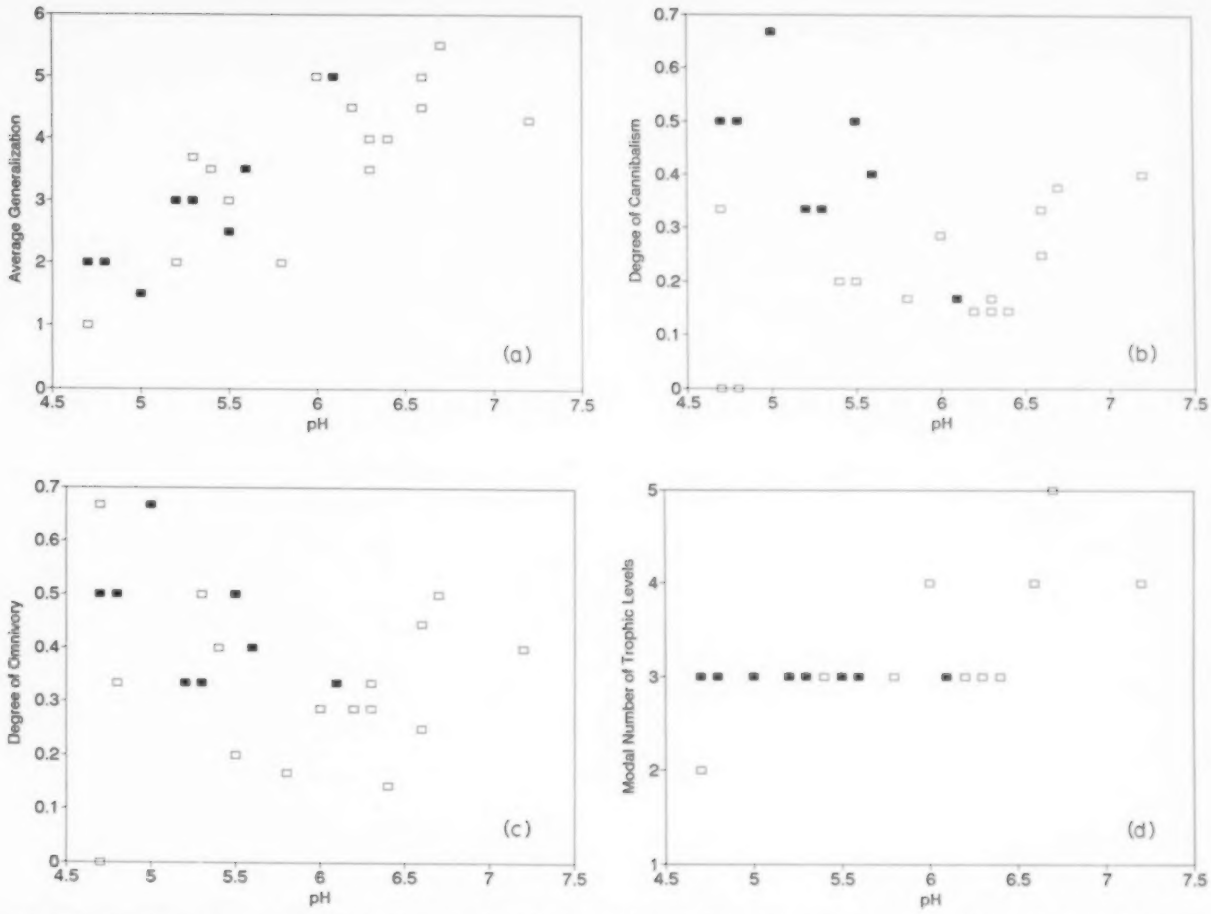

FIG. 3. Food web parameters versus pH. (a) Generalization; (b) degree of cannibalism; (c) degree of omnivory; (d) modal number of trophic levels. Markers are as defined for Fig. 2.

tional level. Pimm (1982) suggested that a testable hypothesis emerges from that view: assuming constant ecological transfer efficiencies, "every order of magnitude increase in primary productivity should permit an additional trophic level." Upon testing the hypothesis by examining food chain lengths in terrestrial and aquatic ecosystems of varying primary productivities (from 10^1 to 10^4 mg $C \cdot m^{-2} \cdot d^{-1}$), Pimm (1982) found that no such relationship existed. Yet he also noted that "at the extreme, low productivities must limit the length of food chains." Oksanen et al. (1981) showed that in freshwater pelagic communities, low productivity can limit food chain length. Because the precipitation-acidified lakes of North America are almost invariably ultraoligotrophic, such a limitation may exist, facilitated by dominance of inedible species among the already scarce producers. Schindler (1990) also suggested that acidification shortens the food chain by eliminating "sensitive species in mid-food chain . . . that have no functional analog" in the ecosystem. Once eliminated, these species are not replaced. Species that fall into this category include *Leptodora kindtii* and *Epischura lacustris*. Both are predators of other zooplankton and food items of planktivorous fish.

In conclusion, the results of this study indicate that acidification markedly affects food web structure. Complexity is greatly reduced, the number of trophic levels declines, and omnivory and cannibalism become more pronounced. Future research is needed to investigate the structural responses of webs in other regions (e.g. Ontario and Scandanavia) to acidification to determine whether the same general patterns emerge. There is especially a need for additional research on food web function, since the structural changes described herein and the results of previous research imply reduced energy flow to higher trophic levels as a result of lake acidification.

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References

BRANDL, Z., AND C. H. FERNANDO. 1978. Prey selection by the cyclopoid copepods *Mesocyclops edax* and *Cyclops vicinus*. Verh. Int. Ver. Limnol. 20: 2505–2510.

CARTER, J. C. H., W. D. TAYLOR, R. CENGALATH, AND D. A. SCRUTON. 1986. Limnetic zooplankton assemblages in Atlantic Canada with special reference to acidification. Can. J. Fish. Aquat. Sci. 42: 444–456.

COHEN, J. E. 1978. Food webs and niche space. Princeton University Press, Princeton, NJ.

COHEN, J. E., AND F. BRIAND. 1984. Trophic links of community food webs. Proc. Natl. Acad. Sci. USA 81: 4105–4109.

CONFER, J. L., T. KAARET, AND G. E. LIKENS. 1983. Zooplankton diversity and biomass in recently acidified lakes. Can. J. Fish. Aquat. Sci. 40: 36–42.

ERIKSSON, M. O. G., L. HENRIKSON, B. I. NILSSON, G. NYMAN, H. G. OSCARSON, AND A. E. STENSON. 1980. Predator–prey relations important for the biotic changes in acidified lakes. Ambio 9: 248–249.

GLIWICZ, Z. M. 1977. Food size selection and seasonal succession of filter feeding zooplankton in an eutrophic lake. Ekol. Pol. 25: 179–225.

HAVENS, K. E. 1989. Seasonal succession in the plankton of a naturally acidic, highly humic lake in northeastern Ohio, U.S.A. J. Plankton Res. 11: 1321–1327.

HAVENS, K. E., AND J. DECOSTA. 1985a. An analysis of selective herbivory in an acid lake and its importance in controlling phytoplankton community structure. J. Plankton Res. 7: 207–222.

1985b. The effect of acidification in enclosures on the biomass and population size structure of *Bosmina longirostris*. Hydrobiologia 122: 153–158.

1987. Freshwater plankton community succession during experimental acidification. Arch. Hydrobiol. 111: 37–65.

HAVENS, K. E., AND R. T. HEATH. 1989. Aid and aluminum effects on freshwater zooplankton: an in situ mesocosm study. Environ. Pollut. 62: 195–211.

1991. Increased transparency due to changes in the algal size spectrum during experimental acidification in mesocosms. J. Plankton Res. 13: 673–679.

HENRIKSON, L., AND H. G. OSCARSON. 1981. Corixids (Hemiptera–Heteroptera), the new top predators in acidified lakes. Verh. Int. Ver. Limnol. 21: 1616–1620.

HUTCHINSON, G. E. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? Am. Nat. 93: 145–159.

JANICKI, A. J., AND J. DECOSTA. 1984. The filtering rates of four herbivorous cladocerans on nanoplankton and net plankton fractions in an acid lake in West Virginia. Int. Rev. Gesamten Hydrobiol. 69: 643–652.

KELLER, W., AND J. R. PITBLADO. 1984. Crustacean plankton in northeastern Ontario lakes subjected to acidic deposition. Water Air Soil Pollut. 23: 271–291.

KERFOOT, W. C. [ED.] 1980. Evolution and ecology of zooplankton communities. American Society of Limnology and Oceanography Special Symposium Volume 3. University Press, Hanover, NH.

KLUGH, B. A., AND H. C. MILLER. 1926. The hydrogen ion concentration range of *Daphnia magna*. Trans. R. Soc. Can. 5: 225–227.

LANE, P. A. 1978. Role of invertebrate predation in structuring zooplankton communities. Verh. Int. Ver. Limnol. 20: 480–485.

LANE, P. A., AND A. BLOUIN. 1985. Qualitative analysis of the pelagic foodwebs of three acid-impacted lakes in Nova Scotia, Canada. Int. Rev. Gesamten Hydrobiol. 70: 203–220.

LINDEMAN, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology 23: 399–418.

LOWNDES, A. G. 1926. Prof. Labbe's production of allomorphs. Nature (Lond.) 118: 551–552.

MACISAAC, H. J., T. C. HUTCHINSON, AND W. KELLER. 1987. Analysis of planktonic rotifer assemblages from Sudbury, Ontario, area lakes of varying chemical composition. Can. J. Fish. Aquat. Sci. 44: 1692–1701.

MILLS, K. H., S. M. CHALANCHUK, L. C. MOHR, AND I. J. DAVIES. 1987. Responses of fish populations in Lake 223 to 8 years of experimental acidification. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 114–125.

NERO, R. W., AND W. G. SPRULES. 1986. Predation by three glacial opportunists on natural zooplankton communities. Can. J. Zool. 64: 57–64.

ODUM, E. P. 1985. Trends expected in stressed ecosystems. Bioscience 35: 419–422.

OKSANEN, L., S. D. FRETWELL, J. ARRUDA, AND P. NIEMELA. 1981. Exploitation ecosystems in gradients of primary productivity. Am. Nat. 118: 240–261.

OVERREIN, L. N. [ED.]. 1980. Acid precipitation — effects on forest and fish. Research Report 19/80. SNSF, Norway. 175 p.

PAINE, R. T. 1988. Food webs: road maps of interactions or grist for theoretical development? Ecology 69: 1648–1654.

PIMM, S. L. 1982. Food webs. Chapman and Hall, London. 219 p.

PIMM, S. L., AND J. H. LAWTON. 1977. Number of trophic levels in ecological communities. Nature (Lond.) 268: 329–331.

1978. On feeding on more than one trophic level. Nature (Lond.) 275: 242–244.

PINEL-ALLOUL, B., G. METHOT, G. VERREAULT, AND Y. VIGNEAULT. 1990. Zooplankton species associations in Quebec lakes: variation with abiotic factors, including natural and anthropogenic acidification. Can. J. Fish. Aquat. Sci. 47: 110–121.

PREPAS, E. 1978. Sugar-frosted *Daphnia*: an improved fixation technique for Cladocera. Limnol. Oceanogr. 23: 557–559.

ROFF, J. C., AND R. E. KWIATKOWSKI. 1977. Zooplankton and zoobenthos of selected northern Ontario lakes of different acidities. Can. J. Zool. 55: 899–911.

SAS INSTITUTE. 1985. SAS user's guide: statistics version 5. SAS Institute, Cary, NC.

SCHINDLER, D. W. 1990. Experimental perturbations of whole lakes as tests of hypotheses concerning ecosystem structure and function. Oikos 57: 25–41.

SCHINDLER, D. W., K. H. MILLS, D. F. MALLEY, D. L. FINDLAY, J. A. SCHEARER, I. J. DAVIES, M. A. TURNER, G. A. LINSEY, AND D. R. CRUIKSHANK. 1985. Long-term ecosystem stress: the effects of years of experimental acidification on a small lake. Science (Wash., DC) 228: 1395–1401.

SCHOENER, T. M. 1989. Food webs from the small to the large. Ecology 70: 1559–1589.

SPRULES, W. G. 1975. Factors affecting the structure of limnetic crustacean zooplankton communities in central Ontario lakes. Verh. Int. Ver. Limnol. 19: 635–643.

SPRULES, W. G., AND J. E. BOWERMAN. 1988. Omnivory and food chain length in zooplankton food webs. Ecology 69: 418–426.

STOKES, P. M., AND Y. K. YUNG. 1986. Phytoplankton in selected LaCloche (Ontario) lakes, pH 4.2–7.0, with special reference to algae as indicators of chemical characteristics, p. 57–72. *In* J. P. Smol, R. W. Batterbee, R. B. Davis, and J. Merilainen [ed.] Diatoms and lake acidity. W. Junk, The Netherlands.

WATRAS, C. J., AND T. M. FROST. 1989. Little Rock Lake (Wisconsin): perspectives on an experimental ecosystem approach to seepage lake acidification. Arch. Environ. Contam. Toxicol. 18: 157–165.

YAN, N. D., AND R. STRUS. 1980. Crustacean zooplankton communities of acidic, metal-contaminated lakes near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. 37: 2282–2293.

Mechanisms of Impact of an Introduced Crayfish (*Orconectes rusticus*) on Littoral Congeners, Snails, and Macrophytes

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Since its introduction 30 yr ago, *Orconectes rusticus* has displaced *O. propinquus* and *O. virilis* and reduced macrophytes in several northern Wisconsin lakes. Since 1973, rate of displacement by *O. rusticus* varied among lakes, but relative abundance of *O. rusticus* increased in all nine lakes sampled in 1988 (eight of which were sampled in 1973 or 1975 and 1980). Using long-term field data and laboratory experiments, we explored mechanisms of differential impact of *O. rusticus* (relative to congeners) on benthic community structure. Displacement of *O. propinquus* (but not *O. virilis*) by *O. rusticus* increased mean crayfish size. Congener feeding preference did not differ among morphologically different macrophytes or between different-sized snails; however, weight-specific consumption and destruction rates did. *Orconectes rusticus* consumed more snails than its congeners and sublethally damaged more snails than *O. virilis*. *Orconectes rusticus* consumed more macrophyte biomass than *O. propinquus*; however, *O. rusticus* did not destroy (consumption plus fragmentation) more macrophyte biomass than its congeners. Changes in benthic community structure after displacement of congeners by *O. rusticus* may result from larger size (which probably lowers resource depression in the presence of size-selective fish predators) and greater weight-specific reductions of prey.

Depuis son introduction trente ans passés, *Orconectes rusticus* a déplacé *O. propinquus* et *O. virilis* et a entraîné une baisse de la biomasse de macrophytes dans plusieurs lacs septentrionaux du Wisconsin. Depuis 1973, le taux de déplacement a varié parmi les neuf lacs expérimentaux échantillonnés en 1988 (dont huit ont été échantillonnés en 1973 ou en 1975 et 1980), mais l'abondance relative de *O. rusticus* a augmenté dans tous ces bassins. À l'aide de données recueillies sur le terrain pendant une longe période et de données expérimentales, les auteurs ont étudié des modes d'impact différentiel de *O. rusticus* (par rapport à ses congénères) sur la structure de la communauté benthique. Le déplacement de *O. propinquus*, mais non de *O. virilis*, par *O. rusticus* a entraîné une augmentation de la longueur moyenne des écrevisses. En ce qui concerne l'alimentation des congénères, leur préférence entre des macrophytes morphologiquement différents ou des escargots de différentes tailles n'était pas différente; toutefois, les taux de consommation et de destruction relatifs au poids l'étaient. *Orconectes rusticus* a consommé plus d'escargots que ses congénères et en a blessés un plus grand nombre que *O. virilis*. De plus, elle a consommé une plus grande biomasse de macrophytes (consommation plus fragmentation) que *O. propinquus*, mais la biomasse qu'elle a détruite n'était pas aussi importante que celle détruite par ses congénères. Les modifications de la structure de la communauté benthique après le déplacement des congénères de *O. rusticus* peuvent être le résultat de la plus grande taille des proies (qui amène probablement une baisse de l'abondance des ressources en présence de poissons recherchant certaines tailles de proies) et d'une baisse plus importante du nombre de proies de certains poids.

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The recent (circa 1960), probably inadvertent introduction of the crayfish, *Orconectes rusticus*, in northern Wisconsin lakes (Capelli and Magnuson 1983) has affected congeneric crayfish and other benthic fauna and flora. At least in the short term, *O. rusticus* has displaced its congeners (*O. propinquus* and *O. virilis*) (Capelli 1982). In addition, anecdotal evidence suggests that *O. rusticus* has a negative impact (relative to its congeners) on the abundance of submersed macrophytes, associated benthic invertebrates and fish in at least seven northern Wisconsin lakes (Lorman 1975; Magnuson et al. 1975; Lorman 1980; Lodge et al. 1985; Lodge and Lorman 1987). Residents and commercial crayfish trappers on these lakes noticed a decline in submersed macrophyte abundance coincident with an increase in crayfish abundance (Lorman 1975). Soon after *O. rusticus* introduction, macrophytes disappeared from Lake Metonga and walleye recruitment ceased (Lodge

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et al. 1985). In Long Lake, visual SCUBA sampling in 1974 indicated that the abundances of submerged macrophytes and *O. rusticus* were inversely related (Magnuson et al. 1975). In in situ cage experiments, *O. rusticus*, at densities not exceeding those found regionally, substantially reduced macrophyte abundance and species richness (Lodge and Lorman 1987).

Mechanisms of differential impact of *O. rusticus* (relative to congeners) on littoral benthic community structure are not well understood but may include higher metabolic rate (Momot 1984), larger individual size, higher population density, and higher population biomass (Lorman 1980; Lodge et al. 1985, 1986). In this study, we document long-term trends in crayfish species displacement and test six potential mechanisms of the apparently greater impact *O. rusticus* has on benthic communities (relative to *O. propinquus* and *O. virilis*).

Because previous studies indicated highly variable among-lake changes in percentage of *O. rusticus* over the short term (Capelli 1982; Capelli and Magnuson 1983) or reversible displacement of *O. virilis* by *O. propinquus* over the long term within one lake (Lodge et al. 1986), we document displacement patterns over a longer time span in several northern Wisconsin lakes. Specifically, we assess current crayfish species composition and abundance in nine lakes, eight of which were previously sampled by Capelli (1982) or Capelli and Magnuson (1983) in 1973 or 1975 and 1980.

We use our long-term field studies and laboratory feeding experiments to test six predictions about mechanisms causing *O. rusticus* to have a greater negative impact than its congeners on the abundance of littoral flora and fauna: (1) After establishment of *O. rusticus*, mean carapace length of crayfish populations is greater. Because susceptibility to fish predation is inversely related to crayfish size (Stein 1977), larger crayfish should forage longer and farther from shelter. (2) After establishment of *O. rusticus*, total (i.e. all species pooled) crayfish population density increases. (3) Likewise, after establishment of *O. rusticus*, total population biomass increases. Assuming similar weight-specific feeding rates, a greater biomass of crayfish would consume a greater biomass of macrophytes and associated benthic invertebrates (Lodge and Lorman 1987). (4) On a weight-specific basis, *O. rusticus* consumes or sublethally damages more snails than its congeners. We chose snails from the invertebrate benthos because they are typically the dominant benthic invertebrate in circumneutral northern Wisconsin lakes (Lodge et al. 1987). (5) Greater selectivity by *O. rusticus* for single-stemmed versus highly branched, prostrate macrophytes would more greatly (relative to congeners) reduce macrophyte biomass. Field enclosure experiments indicate that single-stemmed macrophytes are more susceptible to grazing loss from crayfish than are more prostrate, highly branched forms (Lodge and Lorman 1987). (6) On a weight-specific basis, *O. rusticus* consumes or destroys (consumption plus fragmentation) more macrophyte biomass than *O. propinquus* or *O. virilis*.

Materials and Methods

Field Studies

Nine moderately productive drainage lakes (89–344 ha) in Vilas County, Wisconsin, were sampled for crayfish by trapping (as per Capelli 1982) from mid-July to mid-August 1988. Crayfish were sampled with 18–20 cylindrical, wire mesh minnow traps (21 cm diameter, 3.5–4.5 cm entrance holes) baited with 120 ± 5 g of beef liver. Traps were set in the most favorable habitat available (rocky substrate or woody debris) at 2 m depth, 100 m apart, in mid- to late afternoon and were recovered 20–24 h later. Neither deployment nor recovery time significantly affects total catch of traps set for 24 h (Collins et al. 1983; Lodge et al. 1986). During the trapping period, crayfish were intermolt; Form II comprised only 0.4–12% of the catch.

TABLE 1. Regression equations used to convert carapace length (CL, in millimetres) to live crayfish biomass (B, in grams).

Species	Regression equation	Source
O. rusticus	$B=(1.38\times10^{-4})CL^{3.28a}$	Lorman 1980
		Stein and Murphy 1976[b]
O. propinquus	$B=(3.16\times10^{-4})CL^{3.01c}$	Stein and Murphy 1976
O. virilis	$B=(2.49\times10^{-4})CL^{3.03c}$	Morgan 1987

[a]Equation is for both males and females because differences in biomass between equal carapace length male and female crayfish are non-significant (Stein and Murphy 1976).

[b]Reference contained dry biomass to live biomass conversion (live biomass = 3.73 dry biomass) used to modify dry biomass equation of Lorman (1980).

[c]Regression equations based on length to weight data for male crayfish.

During trap deployment, in all but three lakes, we also deployed 10 square 1.0-m^2 quadrats, one quadrat in every other space between traps. In Birch, Van Vliet, and South Turtle lakes, one quadrat was deployed in each of the first 10 spaces between traps; quadrats here were representative of 50% of the area sampled by traps. In comparisons of visual counts with trap indices of species composition and abundance in these three lakes, we used only the data from the first 11 traps.

On the morning following deployment, we counted adult crayfish species (≥20 mm carapace length) in each quadrat using SCUBA. Moving diagonally from one corner, we carefully removed rocks and other cover to expose crayfish. Because we thoroughly and systematically searched each quadrat, our visual SCUBA surveys accurately sampled crayfish density. After sampling quadrats, traps were retrieved. For each trap, we counted and sexed each crayfish, determined Form for males, and measured carapace length (±0.1 mm) of 200 male crayfish in each species (or all males caught if less than 200).

We tested the reliability of traps as indices for species composition using visual count data as the standard. Only numbers of adult males were used to calculate trap indices of species composition because baited traps are highly selective for adult males (males typically comprise greater than 80% of the trap catch (Capelli 1975; this study)) and female and male trap catches change independently during the year (Lodge et al. 1986).

We then used chi-square to compare 1988 trap indices of species composition with those obtained in 1973 (Capelli and Magnuson 1983), 1975, and 1980 (Capelli 1982). For each year, we determined trap indices of crayfish abundance (male crayfish per trap) and tested their reliability using visual survey results as the standard. Because *O. rusticus* was more trapable than its congeners (see below), we corrected all data (including those previously published by Capelli (1982) and Capelli and Magnuson (1983)) for trapping bias. For all lakes but White Sand (where size was not measured), we determined, by species, a size–frequency distribution for males ≥20 mm carapace length caught in traps in 1975, 1980, and 1988. From

size–frequency distributions, we estimated live crayfish biomass (adult male biomass per trap) based on carapace length to weight regressions (Table 1). Because only total numbers, not catch per trap data, were available for 1973, 1975, and 1980, we tested statistically null hypotheses about change in trap indices of carapace length, abundance, or biomass by determining whether mean values for 1973, 1975, or 1980 fell outside of the 95% confidence range for 1988 data.

We tested whether mean carapace length of crayfish populations increased after establishment of *O. rusticus* (Prediction 1) using estimates, based on trap catches, for 1975, 1980, and 1988. We present only means in this paper, but medians and modes showed the same trends as the means (Olsen 1989). Using trap catches as indices for population abundance, we determined species-specific density and tested if establishment of *O. rusticus* increased total (i.e. all species pooled) crayfish population density (Prediction 2). Likewise, we determined contribution of *O. rusticus* to population biomass and tested whether total population biomass increased after establishment of *O. rusticus* (Prediction 3). Predictions 1–3 were tested in five lakes that were dominated by either *O. propinquus* (Big, Birch, Boulder, and Papoose) or *O. virilis* (Van Vliet) in 1975 but were dominated by *O. rusticus* in 1988. We concluded that the respective prediction was supported if three of the four lakes originally dominated by *O. propinquus* showed the predicted increase in mean carapace length, population density, or population biomass after switching to *O. rusticus* domination.

Laboratory Experiments

Collection and holding of experimental organisms

We collected adult male crayfish with traps as described previously. For tests of crayfish consumption on the hydrobiid snail *Amnicola*, crayfish and snails were housed separately at the University of Notre Dame Environmental Research Center (UNDERC), Land O' Lakes, Wisconsin, in aerated aquaria and exposed to natural summer photoperiod and daily fluctuations in ambient air temperature (water temperature 17–26°C). To eliminate potential intersex variability within the congeners, only males were used. For tests of sublethal damage of *Helisoma* by crayfish and consumption and fragmentation of macrophytes, crayfish, snails, and macrophytes were housed separately in 150-L fiberglass flow-through aerated aquaria (well water temperature 12–15°C) exposed to a 16 h light : 8 h dark photoperiod. Crayfish were fed Purina Fish Chow; snails were fed commercial flake fish food. All experiments were conducted during June–September.

Consumption and damage of snails: testing Prediction 4

We used *Amnicola* spp. as prey to test if *O. rusticus* has a greater weight-specific feeding rate than *O. propinquus* or *O. virilis*. In each replicate of this experiment, three male congeners (± 1 g and of the same Form) were put separately in 45-L aquaria. Crayfish (range of carapace length 31–36 mm) were not used more than once. Aerated water depth was 3.0 cm to keep snails within reach of crayfish. During the first six replicates, water temperature fluctuated with daily ambient air temperature (water temperature 17–26°C). In the remaining replicates (7–16), water temperature was 17.5–18.5°C.

Crayfish were fed 100 *Amnicola*; crayfish were then starved for 24 h and subsequently allowed to feed on 250 *Amnicola* (shell length 2.2–5.3 mm) for 24 h. Snails remaining were counted. We checked crayfish before and after the experiment for signs of molting. If crayfish molted within 4 d of the experiment, data were not used. We used two morphologically similar, but different-sized *Helisoma* congeners (*Helisoma campanulatum* (Say), 6–9 mm diameter; *H. trivolvis* (Say), 10–13 mm diameter) to determine if *O. rusticus* (range of carapace length 31–39 mm) sublethally damaged more snails than its congeners. We used the same protocol as above except that we added a barrier at the air–water interface to prevent *Helisoma* spp. from crawling beyond reach of crayfish. After the feeding trial, we counted the number of snails with damaged shells yet alive.

TABLE 2. Equations used in Experiment C to compute corrected consumption (CC) (consumed macrophyte biomass (grams) corrected for change in macrophyte biomass in control macrophytes).

Equation (1) $\Delta CB = CB_f \div CB_i$

Equation (2) $CC = ((\Delta CB)(EB_i)) - EB_f$

ΔCB = change in live macrophyte biomass in the control (no crayfish) chamber
CB_f = final live macrophyte biomass in control chamber
EB_f = final live macrophyte biomass in experimental chamber
CB_i = initial live macrophyte biomass in control chamber
EB_i = initial live macrophyte biomass in experimental chamber

Consumption and destruction of macrophytes: testing Predictions 5 and 6

We used four macrophyte species, two prostrate and highly branched (*Ceratophyllum demersum* (L.) and *Elodea canadensis* (Michx.)), one rosulate (*Isoetes* spp.), and one single-stemmed (*Potamogeton richardsonii* (Benn)), to test Prediction 5 that *O. rusticus* prefers (relative to congeners) single-stemmed macrophytes and Prediction 6 that *O. rusticus* consumes or destroys (consumption plus fragmentation) more macrophyte biomass than *O. propinquus* or *O. virilis*. We used crayfish (range of carapace length 31–40 mm) and macrophytes within 3 wk of collection.

Each 38-L aquarium was divided with Plexiglas into control (no crayfish) and experimental (one crayfish) halves and had a perforated Plexiglas false floor. Macrophyte species were randomly assigned to perforations. Crayfish shelters were provided in both halves of the tanks. Intermolt male congeners (± 1 g) were starved for 24 h in the experimental chamber and removed. Aerated water level was deep enough at 5.0 cm to cover macrophyte shoots but shallow enough to ensure that no macrophyte tissue was inaccessible to crayfish; temperature was 16–17°C. Macrophytes were spun to constant weight and seven shoots (≤5.0 cm) of each species (4 ± 0.4 g per macrophyte species) were secured to the floor holes with paper clips.

Crayfish were then replaced, allowed to feed for 6 d, and then removed. Attached and detached macrophyte shoots were spun and weighed by species (nearest 0.01 g). Weight change in experimental macrophytes was corrected for mean weight change in control macrophytes (Table 2). In most cases, control macrophytes grew slightly.

For all crayfish, the total macrophyte biomass eaten per crayfish (pooled over all macrophyte species) was less than maximum stomach capacity (based on regression of maximum stomach capacity (grams dry weight) on carapace length for *O. rusticus* (Butler 1983)). Weight-specific differences in congener stomach capacity were assumed to be negligible. Because crayfish did not satiate during the experiment, this suggests that crayfish choice among macrophytes remained independent dur-

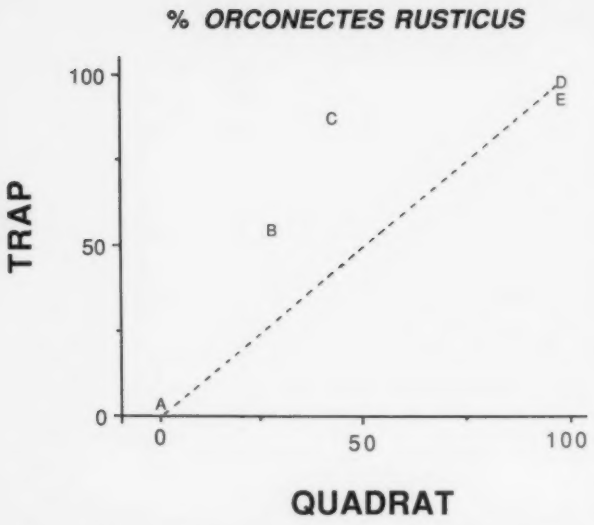


FIG. 1. Relationship between percent *O. rusticus* determined by visual sampling of quadrats and by trapping. Points represent the mean for quadrat (N = 9–10) and trap catches (N = 18–20) in each of nine lakes: White Sand (A), South Turtle (B), Boulder (C), Birch, Island, and Papoose (D), and Big, Clear, and Van Vliet (E). Broken line indicates perfect correlation between trap and quadrat catches.

ing the experiment. Therefore, we use ANOVA to test for differences in selectivity among crayfish congeners.

Results and Discussion

Field Studies

Reliability of trap catches as indices of species composition

Because *O. rusticus* was the only species found in both traps and quadrats in eight of nine lakes sampled, comparison of trap and quadrat catches was based on only one species. Percent *O. rusticus* in traps was well predicted by visual surveys (r^2 = 0.95, $p < 0.001$, N = 9 lakes, 7 df, arcsin $\sqrt{p}$ transform). Because data points for six of nine lakes were clustered (Fig. 1), we also tested significance using df = 2 (as if only four lakes were sampled); with this conservative criterion, $p = 0.05$. Our data do, however, indicate that *O. rusticus* is overrepresented in trap catches. In the two lakes with intermediate abundance of *O. rusticus* population (89 and 56% of trap catch, respectively, in Boulder and South Turtle; Fig. 1), traps apparently overestimated relative abundance of *O. rusticus* by factors of 2.1 and 2.0, respectively. Therefore, to correct for trapping bias in subsequent analyses, we divided trap catch of *O. rusticus* by 2 in each lake to give corrected trap index of species composition. Given that trapping bias probably declines towards both extremes of percentage composition, our correction is conservative.

In a similar study comparing visual survey and trap estimates of species composition in 13 northern Wisconsin lakes, Capelli and Magnuson (1983) found that trap catches were reliable indices of species composition. Our results suggest, however, that in lakes with *O. rusticus*, trapping may exaggerate actual change in species composition over time (species displacement rate). Factors that may contribute to differential species trapability include aggressive interactions (Capelli 1975; Capelli and Munjal 1982), abundance of predatory fishes (Collins et al. 1983), and lunar light intensity (Somers and Stechey 1986). Because *O. rusticus* is generally larger than *O. propinquus* and more aggressive than both its congeners (see Lodge et al. 1985), its trapability may be affected less by predators and light level.

Changes in corrected trap indices of species composition

From 1975 to 1980, relative abundance of *O. propinquus* decreased with regard to *O. rusticus*, while that of *O. virilis* decreased with regard to both congeners (Fig. 2A). However, displacement rate of congeners by *O. rusticus* was highly variable among lakes. For instance, rapid displacements occurred in Birch and Papoose lakes with relative abundance of *O. rusticus* increasing from 6 to 92% and 7 to 100%, respectively. But in South Turtle, *O. rusticus* increased from 5 to only 18%. Despite our more conservative analysis, our conclusions regarding 1975–80 changes are little different from Capelli's (1982) analysis of the same period.

From 1980 to 1988, six of eight lakes had increases in relative abundance of *O. rusticus*, although increases were significant in only four lakes ($p < 0.05$, chi-square; Fig. 2A). In the remaining two lakes, relative abundance of *O. rusticus* either remained 100% (Papoose) or did not change (Birch). As during 1975–80, the rate of displacement of congeners by *O. rusticus* was highly variable among lakes. Between 1973 and 1988, *O. rusticus* invaded White Sand Lake (monospecific for *O. virilis* in 1973; Fig. 2A) and in 1988 comprised <2% of the crayfish species total.

Displacement of congeners by *O. rusticus* is generally consistent with Capelli's hypothesis of competitive exclusion (Capelli 1982; Capelli and Munjal 1982; Berrill 1985). However, variable displacement rate among lakes and reversible displacement of *O. virilis* by *O. propinquus* within one lake (Lodge et al. 1986) suggest that additional community structuring forces, including reproductive interference and differential susceptibility to size-selective fish predation (relative to congeners) (Berrill 1978, 1985; Butler and Stein 1985; Lodge et al. 1985), are probably involved (Lodge et al. 1986). Elucidation of the mechanisms underlying this variable displacement rate among lakes warrants further study.

Our results indicate that *O. rusticus* continues to displace its congeners in many northern Wisconsin lakes. Displacements of congeners (*O. deanae, O. limosus, O. obscurus, O. propinquus, O. sanbornii*, and *O. virilis*) by *O. rusticus* have been documented for other lentic and lotic habitats in Massachusetts (Smith 1981), New Mexico (Bouchard 1978), Ohio (Turner 1926; Flynn and Hobbs 1984; Jezerinac 1982; Butler and Stein 1985; Jezerinac 1986), and Tennessee (Bouchard 1978). Our data suggest that invasion by *O. rusticus* results in long-term establishment of this crayfish as a dominant species in northern Wisconsin lakes.

Reliability of trap catches as indices of crayfish absolute abundance

Trap catches (all species pooled and corrected for twofold greater trapability of *O. rusticus*; see above) were reliable indices of visual survey data (r^2 = 0.71, $p < 0.01$, ANOVA, log–log transform). In another study of five northern Wisconsin lakes, trap catches and visual surveys were significantly correlated (r = 0.97, $p < 0.05$) (Capelli 1975). In a similar study of 19 central Ontario lakes, Collins et al. (1983) found that abundance of predatory fishes affected trapability. Because predatory fish abundance data are unavailable for our study

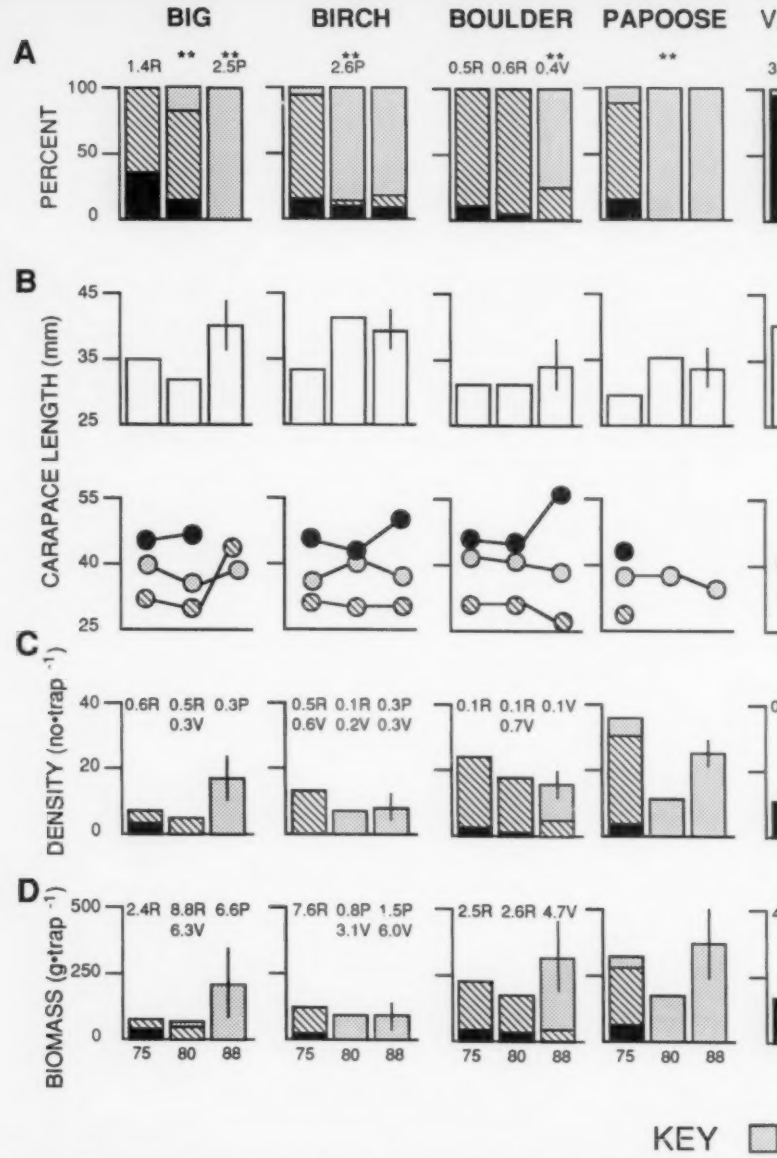
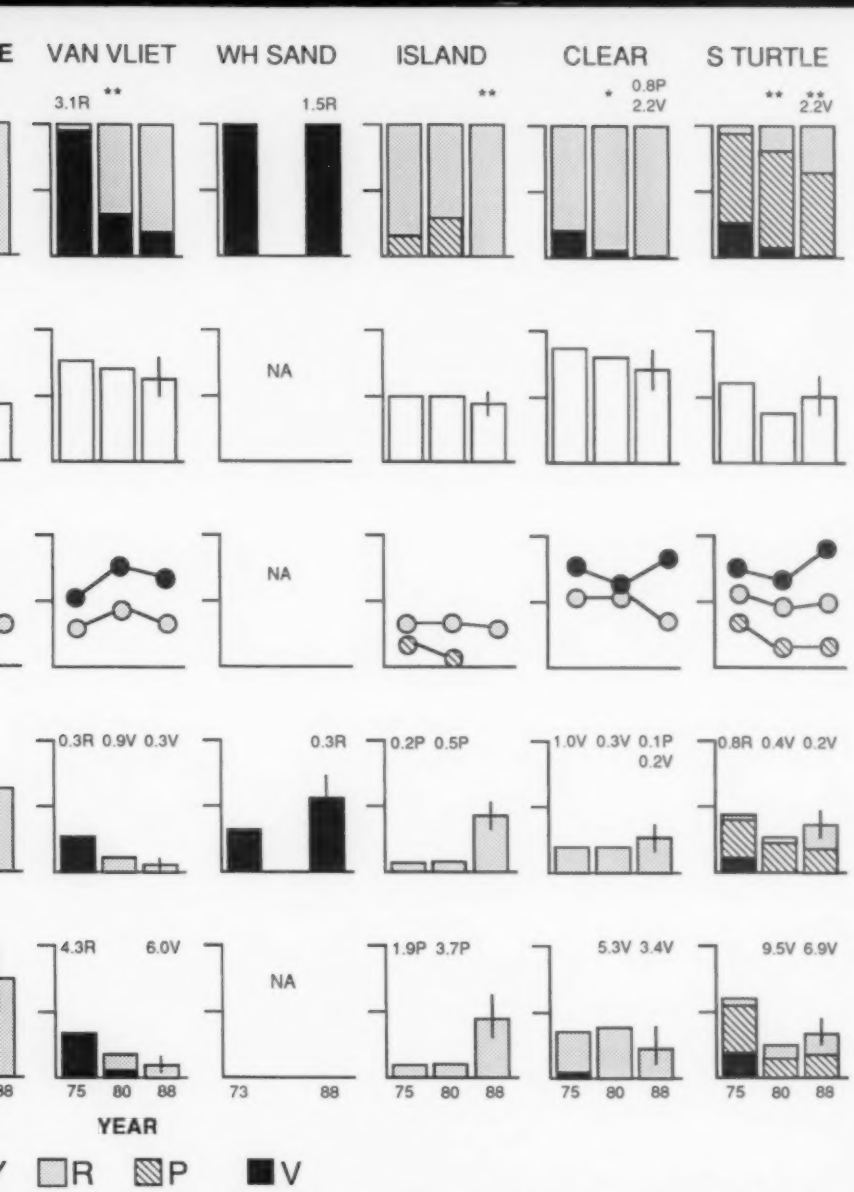


FIG. 2. Changes in corrected trap index of (A) relative abundance, (B) population (top) an
dance, and (D) corrected trap index of biomass (live weight) for adult male crayfish in nin
($*p < 0.05$, $**p < 0.001$, chi-square) from 1975 to 1980. Asterisks above the right bar indica
or <10 (Fig. 2D) are indicated by numbers above the bars. Vertical lines denote 95% con
calculated for the 1973, 1975, and 1980 data because catches per trap were unavailabl
O. propinquus caught in 1988.



(top) and species-specific (bottom) mean carapace length, (C) corrected trap index of absolute abun-
sh in nine northern Wisconsin lakes. Asterisks above the middle bar in Fig. 2A indicate significant changes
r indicate significant changes from 1980 to 1988. Percents <4% (Fig. 2A) and values <1.4 (Fig. 2C)
95% confidence intervals (Fig. 2B–2D , all species combined). Confidence intervals could not be
available. Carapace length data were not available (NA) for White Sand Lake and for the one Clear Lake

lakes, we restricted ourselves to within-lake comparisons and assumed that major changes in predator density did not occur over the span of our survey.

Because trap catches provided estimates similar to visual surveys in this and previous studies, and only trapping data were available for 1973, 1975, and 1980, we used corrected trap catches as indices of crayfish abundance.

Mean crayfish size, density, and population biomass: testing Predictions 1–3

Our data support the prediction that mean crayfish size increases after establishment of *O. rusticus*. In three of the four lakes where *O. rusticus* replaced *O. propinquus* as the dominant crayfish, trap index of mean crayfish size increased (Big, Birch, and Papoose; Fig. 2B, top). However, in the one lake where *O. rusticus* displaced *O. virilis*, mean crayfish size did not change (Van Vliet; Fig. 2B, top). Because carapace length hierarchy was generally *O. virilis* > *O. rusticus* > *O. propinquus* (Fig. 2B, bottom), Prediction 1 was supported when *O. rusticus* displaced *O. propinquus*, but not *O. virilis*.

Patterns in mean size change in the three lakes where there was little change in species composition tend to corroborate that the increase in individual size observed in Big, Birch, and Papoose lakes was linked to the displacement of *O. propinquus* by *O. rusticus*. For example, in lakes dominated by one species from 1975 to 1988 (Island, Clear, and South Turtle), there was no change in individual size of crayfish from 1975 to 1988 (Fig. 2B).

Our results do not support the prediction that crayfish density increases after establishment of *O. rusticus*. In three of four lakes after *O. rusticus* displaced *O. propinquus*, corrected trap indices of crayfish population density (CPD, all species pooled) decreased (Fig. 2C). In one lake (Big Lake), CPD increased after establishment of *O. rusticus* (Fig. 2C). In Van Vliet Lake, where *O. virilis* dominated in 1975, CPD decreased after establishment of *O. rusticus* (Fig. 2C).

However, population biomass did not change after establishment of *O. rusticus*. In only one of the four lakes where *O. rusticus* replaced *O. propinquus* as the dominant crayfish did trap index of crayfish population biomass (CPB, all species pooled) increase (Big; Fig. 2D). In the remaining three lakes that switched from *O. propinquus* to *O. rusticus* domination, CPB did not change (Birch, Boulder, and Papoose; Fig. 2D). In the one lake where *O. rusticus* displaced *O. virilis*, CPB decreased (Van Vliet; Fig. 2D).

In summary, long-term field studies (1973–88) support predictions of increased crayfish size, but not crayfish density or population biomass, when *O. rusticus* displaces *O. propinquus*, but not *O. virilis*. Increase in mean individual crayfish size after *O. rusticus* establishment may result in a greater negative impact on macrophytes and benthic invertebrates when crayfish populations are dominated by *O. rusticus*.

Laboratory Experiments

Consumption and sublethal damage of snails: testing Prediction 4

Congener feeding rates at the two temperature regimes (17–26 and 17.5–18.5°C) did not differ ($p > 0.25$, ANOVA); therefore, only pooled results ($N = 16$) are reported. Results strongly support Prediction 4: weight-specific feeding rate of *O. rusticus* on *Amnicola* was higher than that of *O. propinquus* or *O. virilis* ($p < 0.0001$, ANOVA, Tukey's multiple comparisons; Fig. 3A). For the following reasons, we conclude that

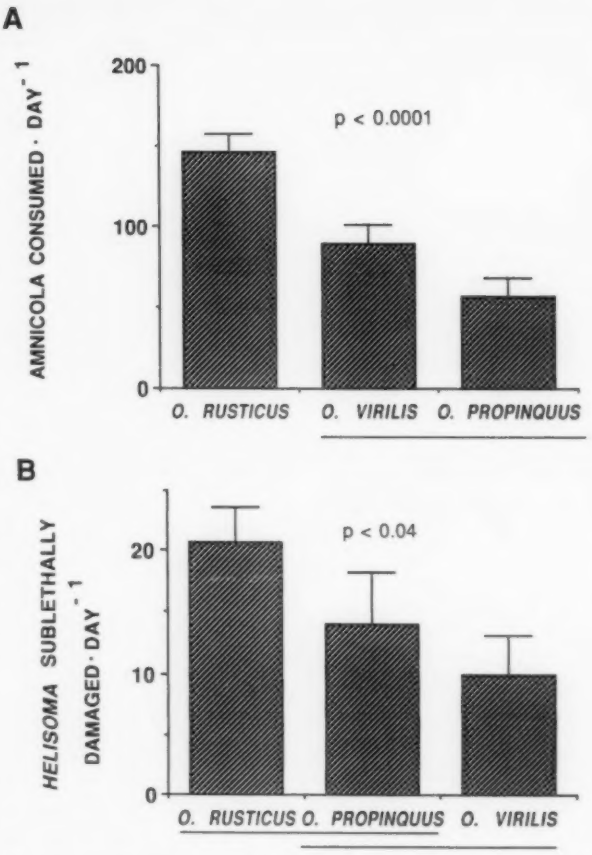


FIG. 3. (A) Consumption of *Amnicola* ($N = 16$) and (B) sublethal damage of *Helisoma* ($N = 8$) by three crayfish congeners during 24-h laboratory experiments. Note the different order of crayfish species on the abscissae. Bars denote one standard error of the mean. Lines connect means that do not differ ($p > 0.05$, ANOVA, Tukey's multiple comparisons).

our results probably accurately reflect the actual hierarchy of congener feeding rates during summer in northern Wisconsin lakes: we conducted experiments with crayfish collected ≤10 d before from northern Wisconsin lakes and exposed them to natural summer photoperiod and temperature. *Amnicola* spp. are among the natural prey of northern Wisconsin crayfish (see Lodge and Lorman 1987) and are abundant in northern Wisconsin lakes.

Because handling time for crayfish on hydrobiid snails was <10 s per snail (pers. obs.), it is improbable that interspecific differences in handling time accounted for the differences in feeding rate among congeners. *Orconectes rusticus* has a higher metabolic rate than *O. virilis* at 17–25°C (Lippson 1976; Momot 1984), which would account for its greater feeding rate relative to *O. virilis*. However, *O. propinquus*, has a higher metabolic rate than *O. rusticus* (Lippson 1976) and this does not support our feeding rate results. However, such comparisons are limited because Lippson (1976) did not report the range of crayfish sizes used and whether his replicates were run using equal-biomass crayfish. Regardless of the mechanism contributing to differences in ingestion rate among *Orconectes* congeners, our experiment strongly suggests that greater weight-specific inges-

tion rate by *O. rusticus* may result in greater impact on the abundance of aquatic snails in northern Wisconsin lakes.

Likewise, Prediction 4 is supported: *O. rusticus* sublethally damaged more *Helisoma* spp. than *O. virilis* ($p < 0.04$, ANOVA, Tukey's multiple comparisons; Fig. 3B). Sublethal snail damage rate was not confounded by differences in selectivity among crayfish congeners for the two *Helisoma* species (snail species effect, $p = 0.67$; crayfish × snail interaction effect, $p = 0.55$, ANOVA). We used two sizes of *Helisoma* congeners because the experiment was designed originally to test crayfish selection between the two morphologically similar (yet different-sized) snails. High encounter rates in our experiment (relative to those in lakes) may have increased the frequency of sublethal damage. In any case, this sort of sublethal damage of snails by crayfish (i.e. removal of shell whorl on planorbid snails) probably does not lower snail fitness (Stahl and Lodge 1990).

Consumption and destruction of macrophytes: testing Predictions 5 and 6

Crayfish consumed and destroyed macrophytes selectively (macrophyte effect, $p < 0.001$, df = 3 in each case); all three congeners showed the same selectivity among macrophyte species for both consumption (crayfish × macrophyte interaction effect, $p = 0.28$, df = 6) and destruction (crayfish × macrophyte interaction effect, $p = 0.88$, df = 6). Highly branched *Ceratophyllum* and *Elodea* were as susceptible to grazing loss as single-stemmed *P. richardsonii*. *Isoetes* was consumed and destroyed the least, with no difference existing among the other three plant species for either consumption or destruction (Tukey's multiple comparisons) (Fig. 4).

In this and previous experiments, *Orconectes* crayfish graze selectively (Lodge 1991), reducing macrophytes in both the laboratory (Seroll and Coler 1975; Lorman 1980) and the field (Lodge and Lorman 1987). In situ enclosure experiments suggest that macrophyte morphology can affect susceptibility to grazing loss: single-stemmed macrophytes appear more susceptible to destruction than rosulate or highly branched forms (Lodge and Lorman 1987). In contrast with the Lodge and Lorman (1987) field results and Prediction 5, greater impact of *O. rusticus* on macrophyte abundance did not result from greater selectivity by *O. rusticus* for the single-stemmed (*P. richardsonii*) macrophyte species.

Because congener feeding rate on macrophytes was not confounded by macrophyte species selection among crayfish congeners (see above), this experiment also tested Prediction 6. Crayfish congeners differed in the amount of macrophyte biomass consumed (*O. rusticus* > *O. propinquus*; Fig. 4A) and destroyed (consumption plus fragmentation: *O. virilis* > *O. propinquus*; Fig. 4B). Total destruction is probably the most relevant measure of crayfish impact on macrophyte abundance in northern Wisconsin lakes. On a weight-specific basis, *O. virilis* is just as destructive of macrophytes as *O. rusticus*.

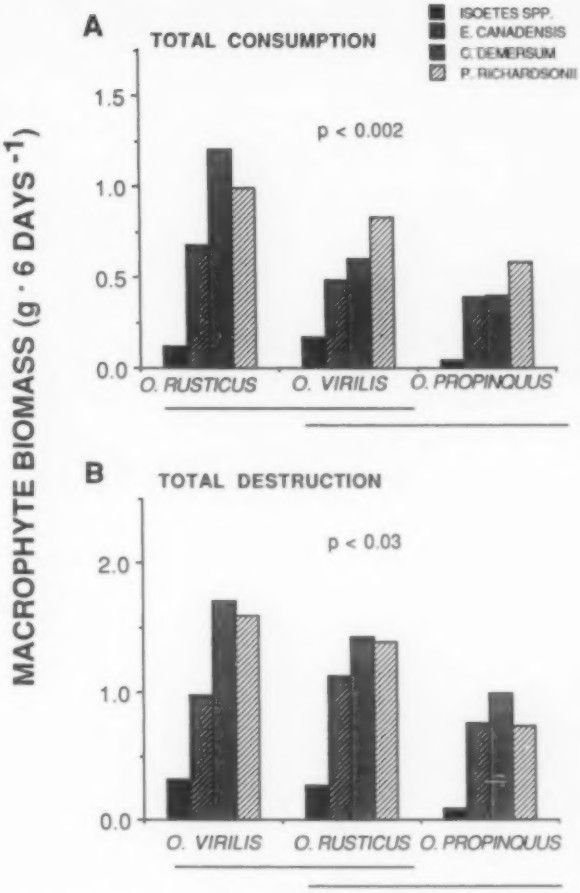


FIG. 4. (A) Total consumption and (B) total destruction (consumption plus fragmentation) of four macrophyte species by three crayfish congeners during a 6-d laboratory selection experiment. Note the different order of crayfish species on the abscissae of each graph. Lines connect crayfish that do not differ in total consumption or total destruction ($p > 0.05$, $N = 9$, ANOVA, Tukey's multiple comparisons).

General Discussion

Our 1973–88 field data indicate that *O. rusticus* tends to displace congeners and becomes the numerically dominant species. During 1973–88, rate of displacement by *O. rusticus* varied among lakes, but relative abundance of *O. rusticus* increased in all nine lakes studied. Successful establishment of *O. rusticus* in northern Wisconsin lakes probably results from superior size-specific competitive ability (relative to *O. propinquus* and *O. virilis*) (Capelli and Munjal 1982) in combination with lower susceptibility to size-selective fish predation (Stein 1977; Berrill 1978; Capelli 1982; Capelli and Munjal 1982; Capelli and Magnuson 1983; see Lodge et al. 1985; Butler and Stein 1985; Lodge et al. 1986). Our long-term data suggest that invasions by *O. rusticus* do not follow the typical "boom and bust" pattern for introduced species (see Simberloff 1981; Williamson and Brown 1986). Whether other lotic and lentic systems recently invaded by *O. rusticus* will show a similar long-term pattern of differential impact by *O. rusticus* on congeners and littoral benthic community structure awaits further study.

We investigated six mechanisms underlying the greater negative impact of *O. rusticus* (relative to congeners) on the abundance of littoral macrophytes and associated benthic invertebrates in northern Wisconsin lakes. Results were at least partly consistent with Predictions 1, 4, and 6 but contradicted Predictions 2, 3, and 5 (Table 3). Our results suggest that on a per biomass basis, a population of *O. rusticus* would produce a

TABLE 3. Summary of test results for mechanisms governing the apparently greater impact of *O. rusticus* (*Or*) (relative to *O. propinquus* (*Op*) and *O. virilis* (*Ov*) on benthic community structure. Four lakes served to test mechanisms 1–3 when *Or* displaced *Op* and one lake when *Or* displaced *Ov* (see Fig. 2).

Mechanisms	Re *O. propinquus*	Re *O. virilis*
1. Individual size: $Or > Ov > Op$	$Or > Op$	$Or < Ov$
2. Population density: $Or > Op > Ov$	$Or < Op$	$Or < Ov$
3. Population biomass: $Or > Ov > Op$	$Or < Op$	$Or < Ov$
4. Weight-specific consumption (*C*) and sublethal damage (*D*) of snails: $Or > Op, Ov$	*C*: $Or > Op$ *D*: $Or = Op$	$Or > Ov$ $Or > Ov$
5. Weight-specific selection for single-stemmed macrophytes: $Or > Op, Ov$	$Or = Op$	$Or = Ov$
6. Weight-specific consumption (*C*) and destruction (*D*) of macrophytes: $Or > Ov, Op$	*C*: $Or > Op$ *D*: $Or = Op$	$Or = Ov$ $Or = Ov$

greater impact on the abundance of benthic flora and fauna than an equal population of its congeners. However, the magnitude of impact on the benthic community after establishment of *O. rusticus* may depend on which congener *O. rusticus* displaces. Field data suggest that in three of four lakes that switched from *O. propinquus* to *O. rusticus* domination during 1975–88, crayfish are larger. Larger size would allow *O. rusticus* to forage longer with impunity from size-selective fish predators (see Stein 1977) over a wider range of substrates (being less restricted to protective cobble shelter) (see Lodge et al. 1985). This impact is intensified by the significantly greater weight-specific ingestion rate of *O. rusticus* (on snails and macrophytes) compared with *O. propinquus* shown in our laboratory experiments.

When *O. rusticus* displaced *O. virilis* ($N = 1$ lake), however, crayfish size decreased (Table 3). In laboratory experiments, *O. rusticus* sublethally damaged more snails than *O. virilis*, and *O. rusticus* had a greater weight-specific ingestion rate than *O. virilis* on snails but not macrophytes. Given data from only one lake, and the potentially counteracting mechanisms, it is difficult to predict what impact on littoral benthos a switch from *O. virilis* to *O. rusticus* entails.

Additional laboratory experiments could further clarify mechanisms of differential impact of *O. rusticus* on benthic community structure. For example, feeding separately housed crayfish congeners, snails, and macrophytes simultaneously in controlled laboratory experiments would elucidate the effect alternative resources have on each congener's rate of macrophyte and invertebrate consumption (see Covich 1977). For example, an alternative food (beef liver) significantly reduced consumption and nonconsumptive destruction of the macrophyte *Vallisneria* by *O. rusticus* (Lorman 1980). The presence of snails probably reduces macrophyte consumption by *O. rusticus* in the littoral zone of northern Wisconsin lakes (Lodge and Lorman 1987).

To ensure that our comparative feeding rate experiments were unbiased, we should ideally have collected all crayfish congeners from one lake or from lakes of similar predation risk. However, we could not trap sufficient numbers of all three crayfish congeners from any one lake. In laboratory behavioral studies, for at least 3 wk after collection, *O. propinquus* from a lake with high bass density were generally less active and more shelter-bound than crayfish from a lake without bass (Collins et al. 1983). In our experiments on snail and macrophyte consumption in which this could have been a problem, we used crayfish 1–18 d from date of collection. Our crayfish source lakes, unlike those of Collins et al. (1983), all contain bass (Black et al. 1963; Olsen 1989); therefore, all our experimental crayfish probably demonstrated some degree of predator-induced resource depression. We could not, however, determine a rank order of predation risk for our crayfish source lakes because predatory fish abundance data are unavailable.

In conclusion, long-term field data from nine northern Wisconsin lakes indicate that invasions by *O. rusticus* tend to result in the displacement of congeners and its long-term dominance; however, displacement rate among lakes is highly variable. Field and laboratory studies support three mechanisms (Table 3) that additively help explain the greater declines in macrophytes and benthic invertebrate abundance in lakes where *O. rusticus* has displaced its congeners: (1) larger size (which probably results in less behavioral inhibition of feeding in the presence of size-selective fish predators), (2) greater weight-specific consumption of snails, and (3) greater weight-specific consumption of macrophytes.

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References

BERRILL, M. 1978. Distribution and ecology of crayfish in the Kawartha lakes of southern Ontario. Can. J. Zool. 56: 166–177.

1985. Laboratory induced hybridization of two crayfish species, *Oconectes rusticus* and *O. propinquus*. J. Crustacean Biol. 5: 347–349.

BLACK, J. J., L. M ANDREWS, AND C. W. THREINEN. 1963. Surface water resources of Vilas County. Wisconsin Conserv. Dep. Publ., Madison, WI. 317 p.

BOUCHARD, R. W. 1978. Taxonomy, distribution and general ecology of the genera of North American crayfishes. Fisheries 3: 11–16.

BUTLER, M. J. 1983. An analysis of replacement mechanisms governing range expansion in crayfish. MS thesis, The Ohio State University, Columbus, OH. 82 p.

BUTLER, M. J., AND R. A. STEIN. 1985. An analysis of mechanisms governing species replacements in crayfish. Oecologia 66: 168–177.

CAPELLI, G. M. 1975. Distribution, life history and ecology of crayfish in northern Wisconsin with emphasis on *Oroconectes propinquus* (Girard). Ph.D. thesis, University of Wisconsin, Madison, WI. 215 p.

1982. Displacement of northern Wisconsin crayfish by *Orconectes rusticus* (Girard). Limnol. Oceanogr. 27: 741–745.

CAPELLI, G. M., AND J. J MAGNUSON. 1983. Morphoedaphic and biogeographic analyses of crayfish distribution in northern Wisconsin. J. Crustacean Biol. 3: 548–564.

CAPELLI, G. M., AND B. J. MUNJAL. 1982. Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus *Orconectes*. J. Crustacean Biol. 2: 486–492.

COLLINS, N. C., H. H. HARVEY, A. J. TIERNEY, AND D. W. DUNHAM. 1983. Influence of predator density on trapability of crayfish in Ontario lakes. Can. J. Fish. Aquat. Sci. 40: 1820–1828.

COVICH, A. P. 1977. How do crayfish respond to plants and mollusca as alternate food resources? Freshwater Crayfish 3: 165–179.

FLYNN, M. F., AND H. H. HOBBS. 1984. Parapatric crayfishes in southern Ohio: evidence of competitive exclusion?. J. Crustacean Biol. 4: 382–389.

JEZERINAC, R. F. 1982. Life-history notes and distributions of crayfishes (Decapoda: Cambaridae) from the Chagrin River basin, northeastern Ohio. Ohio J. Sci. 82: 181–192.

1986. Endangered and threatened crayfishes (Decapoda: Cambaridae) of Ohio. Ohio J. Sci. 86: 177–180.

LIPPSON, R. L. 1976. The distribution of the crayfishes of Michigan with aspects of their life cycle and physiology. Ph.D. thesis, Michigan State University, Lansing, MI. 146 p.

LODGE, D. M. 1991. Herbivory on freshwater macrophytes. Aquat. Bot. (In press)

LODGE, D. M., A. L. BECKEL, AND J. J. MAGNUSON. 1985. Lake-bottom tyrant. Nat. Hist. 94: 32–37.

LODGE, D. M., K. M. BROWN, S. P. KLOSIEWSKI, R. A. STEIN, A. P. COVICH, B. K. LEATHER, AND C. BRONMARK. 1987. Distribution of freshwater snails: spatial scale and the relative importance of physicochemical and biotic factors. Am. Malacol. Bull. 5: 73–84.

LODGE, D M., T. K. KRATZ, AND G. M. CAPELLI. 1986. Long-term dynamics of three crayfish species in Trout Lake, Wisconsin. Can. J. Fish. Aquat. Sci. 43: 993–998.

LODGE, D. M., AND J. G. LORMAN. 1987. Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. Can. J. Fish. Aquat. Sci. 44: 591–597.

LORMAN, J. G. 1975. Feeding and activity of the crayfish *Orconectes rusticus* in a northern Wisconsin lake. MS thesis, University of Wisconsin, Madison, WI. 56 p.

1980. Ecology of the crayfish *Orconectes rusticus* in northern Wisconsin. Ph.D. thesis, University of Wisconsin, Madison, WI. 227 p.

MAGNUSON, J. J., G. M. CAPELLI, J. G. LORMAN, AND R. A. STEIN. 1975. Consideration of crayfish for macrophyte control, p. 66–74. *In* P. L. Brezonik and J. L. Fox [ed.] The proceedings of a symposium on water quality management through biological control. Rep. No. ENV 07-75-1, University of Florida, Gainesville, FL.

MOMOT, W. T. 1984. Crayfish production: a reflection of community energetics. J. Crustacean Biol. 4: 35–54.

MORGAN, G. E. 1987. Population dynamics of an exploited population of *Orconectes virilis* in northwestern Ontario. M.S. thesis, Lakehead University, Thunder Bay, Ont.

OLSEN, T. M. 1989. Impact of the introduced crayfish, *Orconectes rusticus*, in northern Wisconsin lakes: field and laboratory studies. M.S. thesis, University of Notre Dame, Notre Dame, IN. 114 p.

SEROLL, A., AND R. A. COLER. 1975. Demonstrated food preferences of *Orconectes immunis* (Hagen) (Decapoda, Astacidea). Crustaceana 29(3): 319–320.

SIMBERLOFF, D. 1981. Community effects of introduced species, p. 53–81. *In* M. H. Nitecki [ed.] Biotic crises in ecological and evolutionary time. Academic Press, New York, NY.

SMITH, D. G. 1981. Evidence for hybridization between two crayfish species (Decapoda: Cambaridae: *Orconectes*) with a comment on the phenomenon in Cambarid crayfish. Am. Midl. Nat. 105: 405–407.

SOMERS, K. M., AND D. P. M. STECHEY. 1986. Variable trapability of crayfish associated with bait type, water temperature and lunar phase. Am. Midl. Nat. 116: 36–44.

STAHL, T., AND D. M. LODGE. 1990. Effect of experimentally induced shell damage on mortality, reproduction and growth in *Helisoma trivolvis* (Say, 1816). Nautilus 104: 92–95.

STEIN, R. A. 1977. Selective predation, optimal foraging and the predator–prey interaction between fish and crayfish. Ecology 58: 1237–1253.

STEIN, R. A., AND M. L. MURPHY. 1976. Changes in proximate composition of the crayfish *Orconectes propinquus* with size, sex, and life stage. J. Fish. Res. Board Can. 33: 2450–2458.

TURNER, C. L. 1926. The crayfishes of Ohio. Ohio Biol. Surv. Bull. 3: 145–196.

WILLIAMSON, M. H., AND K. C. BROWN: 1986. The analysis and modelling of British invasions. Philos. Trans. R. Soc. Lond. B 314: 505–522.

Interpretation of Otolith Microstructure in Juvenile Winter Flounder (*Pseudopleuronectes americanus*): Ontogenetic Development, Daily Increment Validation, and Somatic Growth Relationships[1]

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Sogard, S. M. 1991. Interpretation of otolith microstructure in juvenile winter flounder (*Pseudopleuronectes americanus*): ontogenetic development, daily increment validation, and somatic growth relationships. Can. J. Fish. Aquat. Sci. 48: 1862–1871.

In winter flounder (*Pseudopleuronectes americanus*), sagittae developed secondary origins of calcium carbonate deposition during metamorphosis just prior to completion of eye migration. Sagittae and lapilli of larvae were bilaterally symmetrical, but those of postmetamorphic individuals showed increasing morphological asymmetry between the left and right side. In juveniles marked with oxytetracycline and maintained in field enclosures for 10 d, increment deposition on sagittae was daily if somatic growth following marking was good (>0.25 mm·d^{-1}), but less than daily in individuals with poor or negative somatic growth (<0.25 mm·d^{-1}). Narrowly spaced increments or divergence of otolith growth from the main rostral–postrostral growth axis, where counts were made, may have limited detection of daily deposition. Lack of detectable daily increments occurred primarily in larger juveniles (>50 mm total length), which had lower absolute growth rates than newly settled juveniles. In oxytetracycline-marked fish there was a significant correspondence between otolith growth and somatic growth in both length and weight. The strength of this relationship, which varied with the specific radius used, was highest (r = 0.854) for the rostral radius of the left sagitta; increment widths along this radius are reliable estimators of prior somatic growth rates.

Chez la plie rouge (*Pseudopleuronectes americanus*), la sagitta est le siège de dépôts de carbonate de calcium d'origine secondaire durant la métamorphose, juste avant la fin de la migration de l'oeil. La sagitta et le lapillus des larves présentent une symétrie bilatérale, mais chez les sujets ayant passé la métamorphose, elles présentent entre le côté gauche et le côté droit une asymétrie morphologique croissante. Chez des juvéniles marqués à l'oxytétracycline et gardés 10 jours en enceintes sur le terrain, le dépôt d'accroissement dans la sagitta était quotidien si la croissance somatique après le marquage était suffisante (>0,25 mm par jour) et moins que quotidien chez les sujets dont la croissance somatique était médiocre ou négative (<0,25 mm par jour). Il se peut que la détection du dépôt quotidien ait été limitée par le fait que les accroissements étaient peu espacés ou que le développement des otolithes s'est fait dans un plan divergent de l'axe principal de croissance rostral–postrostral, là où les comptages ont été faits. Les cas où il n'y a pas eu d'accroissement quotidien détectable ont surtout été vus chez les juvéniles de grande taille (>50 mm longueur totale), le taux de croissance absolu étant plus faible chez ces sujets que chez ceux qui commençaient le stade juvénile. Chez les poissons marqués à l'oxytétracycline, on a constaté une correspondance significative entre le développement des otolithes et la croissance somatique en longueur et en poids. Le degré de certitude de cette relation, qui variait selon le rayon utilisé, était maximal (r = 0,854) dans le cas du rayon rostral de la sagitta de gauche; les largeurs d'accroissement sur ce rayon sont donc des facteurs d'estimation fiables du taux de croissance somatique antérieure.

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Patterns in otolith microstructure of larval and juvenile fishes are widely and profitably used to characterize life history traits. Increment deposition has been demonstrated to be daily in a broad variety of species, allowing precise aging of individuals (see Campana and Neilson 1985 for a recent review). The ability to estimate individual growth histories is supported by several experimental studies demonstrating a significant correspondence between increment widths and somatic growth in length or weight (Wilson and Larkin 1982; Volk et al. 1984; Neilson and Geen 1985; Bradford and Geen 1987; Alhossaini and Pitcher 1988; Radtke 1989). Such age and growth information at the individual level provides the fundamental tools for demographic analysis of fish populations.

The left and right otoliths of most teleosts are largely symmetric to each other in shape, and either may be used in aging studies (Campana and Neilson 1985). Adult flatfishes, however, are characterized by varying degrees of morphological contrast between the left and right sagitta (Smith and Daiber 1977; Nolf 1985). Such bilateral asymmetry is perhaps more likely in flatfishes, with their unique anatomical transformations associated with eye migration during metamorphosis. Some skeletal and neurological structures shift in position to accommodate the migrating eye (Graf and Baker 1988; Ahlstrom et al. 1984), but the otoliths do not. Thus, when the flounder assumes its 90° rotation in body orientation, the flat,

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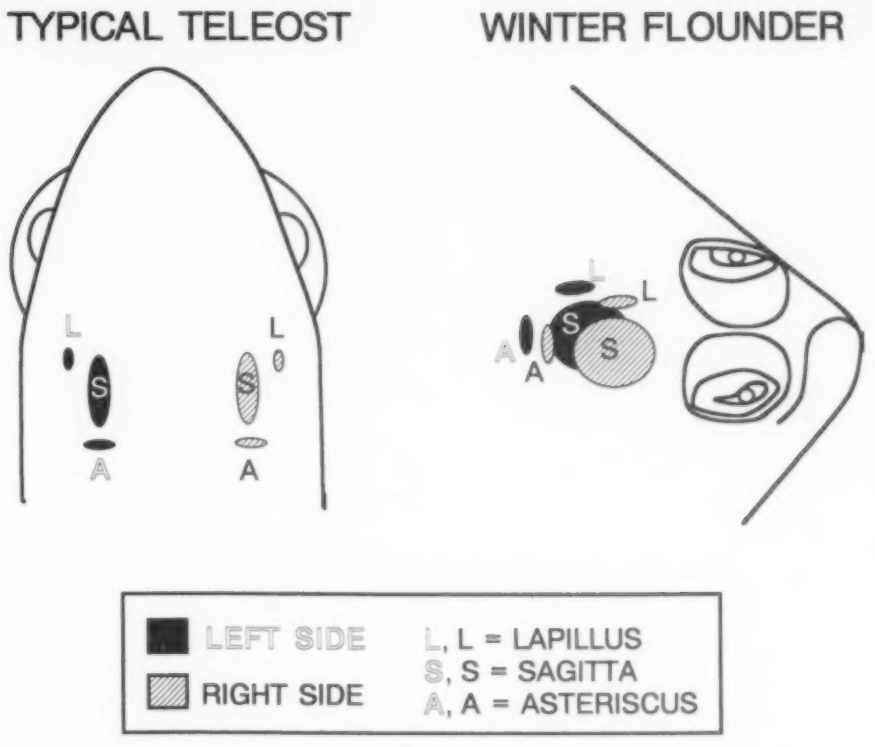

FIG. 1. Top view of the position of the otoliths in a typical teleost and in a winter flounder. The fish are shown in their usual orientation relative to the substrate.

sagittal faces of the otoliths are parallel to the benthos and spatially oriented with one on top of the other (Fig. 1).

In addition to information on age and growth of individual fish, the timing of specific events has also been inferred from otolith microstructure. Life history transitions, such as hatching or metamorphosis, often result in changes in otolith deposition, and thus a visible record of the transition event. A particularly striking change in otolith deposition occurs in the starry flounder (*Platichthys stellatus*) at metamorphosis (Campana 1984a). Calcium carbonate deposition shifts to new foci on the outer surface of the larval otolith, resulting in secondary growth centres. Deposition emanating from the primary growth centre (central primordium) continues until this region is enclosed by growth from these secondary centres (also known as accessory primordia or peripheral nuclei). A similar pattern of development occurs in sagittae of European plaice (*Pleuronectes platessa*) (Alhossaini et al. 1989).

The use of otolith growth to infer past fish growth relies on establishment of a functional relationship between the width of daily increments and the daily somatic growth. Detailed information on this relationship is critical to determining the precision of back-calculated lengths-at-age, but direct comparison of otolith and somatic growth in individuals is rarely accomplished (Wilson and Larkin 1982; Bradford and Geen 1987; Alhossaini and Pitcher 1988). Several studies have shown the relationship between otolith growth and somatic growth to be allometric in response to varying environmental conditions. This uncoupling results in relatively large otoliths in fish with poor somatic growth (Mosegaard et al. 1988; Reznick et al. 1989; Secor and Dean 1989; Secor et al. 1989; Wright et al. 1990). Species-specific differences in the scaling of otolith to somatic growth will affect the reliability of increment widths as estimators of prior growth rates.

In the present study, I describe the development of secondary growth centres and subsequent bilateral asymmetry in transforming winter flounder (*Pseudopleuronectes americanus*) and evaluate the question of daily deposition of increments emanating from these secondary centres. In addition, I directly test the correspondence of otolith growth to somatic growth over short (10 d) time periods, using individually marked juveniles enclosed in natural habitats. These results are used to evaluate the utility of otoliths in estimating ages and growth rates of winter flounder from natural populations.

Materials and Methods

Sagittae from a series of premetamorphic larvae through late young-of-the-year winter flounder were examined to determine the time of development of secondary growth centres. Larvae were collected with a 1-m-diameter, 1-mm-mesh plankton net fished weekly on night flood tides in Little Sheepshead Creek, New Jersey. Larvae used for otolith analysis occurred in plankton collections from April 18 through May 31, 1989. Juveniles were collected by push net, throw trap, and bait trawl from a variety of shallow habitats (<2 m) throughout the summers of 1988 and 1989 in the Little Egg Harbor and Great Bay estuaries of New Jersey.

Larvae were classified into five arbitrary stages based on the degree of migration of the left eye: stage 1 = eyes bilaterally symmetric; stage 2 = left eye just visible from right side; stage 3 = left eye directly on top of dorsum; stage 4 = left eye just past centre of dorsum; stage 5 = eye migration complete. Stage 5 individuals were fully metamorphosed and distinguished from epibenthic juveniles only on the basis of capture by plankton nets. Both sagittae of randomly selected fish in each stage were excised, placed in immersion oil, and examined under a microscope at 100× for the presence of secondary growth centres. Sagittae from epibenthic juveniles were embedded in Spurr resin and polished in the sagittal plane to the central primordium on both sides, using a series of 400–1500 grit sandpaper and alumina powder (0.3 μm).

Two radii on each juvenile sagitta were measured to provide quantitative analysis of left–right asymmetry. These were from the central primordium to the most anterior part of the rostrum and from the central primordium to the opposite, postrostrum edge. Measurements were made with an image analysis system attached to an Olympus BH-2 microscope, using a magnification on the monitor of 160 or 410× depending on the size of the otolith. The relationship between otolith radial measurements and length (SL) of the fish was determined by regression analysis. The slopes of these regressions were then compared between left and right otoliths to test for asymmetry. Two additional measures of potential asymmetry, the total length of the sagittae and the total number of postmetamorphic increments (those emanating from the innermost secondary growth centre), were compared between left and right with paired comparisons *t*-tests.

A test of the rate of increment deposition involved matching the number of increments following a tetracycline-induced fluorescent mark on the otolith with the actual number of days elapsed. Juvenile flounders (22–84 mm total length (TL), $n = 216$) were immersed for 24 h in a 500 $mg \cdot L^{-1}$ solution of oxytetracycline dihydrate in natural seawater (20–25 ppt) diluted with distilled water to about 17 ppt. Immediately following immersion, the flounders were measured, weighed, and individually marked with a small injection of acrylic paint. They were then transferred to 1-m^2 cages in the field (depth at low tide = 30–40 cm) for experiments comparing growth among different estuarine habitats (Sogard 1990). The cage design consisted of a wood frame with stiff plastic mesh (3 mm in diameter) on the sides (46 cm high) and top and galvanized steel edges (5 cm deep) around the bottom for embedding in the sediment; they were open on the bottom to allow foraging on natural substrates. The fish were recovered from the cages after 10 d, measured, weighed, and sagittae removed and cleaned.

The number of increments following the tetracycline mark was counted under light microscopy at 400–1000× magnification. To provide an unbiased count of rings past the mark, 10 rings in from the otolith edge were counted under incandescent light. The UV light was then turned on, the position of the tetracycline-marked increment detected by epifluorescence, and the discrepancy in increment counts by the two methods noted. Counts were made along the longitudinal axis from the outer edge of both the rostrum and postrostrum. It was assumed that increment detection and differences in increment widths would be greatest on the longest axis of the otolith. Because fluorescence from the tetracycline mark was usually very distinct in whole otoliths but was faint or unclear in otoliths that had been embedded and polished, all counts were initially made on whole otoliths in immersion oil. Increments on each otolith were counted on three different dates by the same reader and the results were averaged; the reader did not know the source of the otolith or results of the previous counts.

TABLE 1. Percentage of winter flounder larvae with secondary growth centres in the left or right sagitta. Developmental stage refers to position of migrating eye (see text).

Developmental stage	Size range (mm SL)	Percent with secondary centres		*n*
		Left	Right	
1	4.5–9.6	0	0	7
2	6.0–8.1	0	0	11
3	5.7–7.9	27	32	22
4	6.2–8.3	44	56	16
5	6.5–8.2	100	100	6

Confirmation of these increment counts was subsequently attempted with scanning electron microscope (SEM) examination. For SEM analysis, sagittae were ground and polished in the sagittal plane, etched with 2% EDTA (pH 7.4) for 1–5 min, and sputter coated with gold/palladium. The position of the tetracycline-marked increment was determined by matching with photomicrographs taken under UV illumination.

Otoliths from oxytetracycline-marked fishes held in field cages were also used to determine the relationship between otolith and somatic growth. The distance from the fluorescent mark to the edge of the sagitta was measured for the four radii of right rostrum, right postrostrum, left rostrum, and left postrostrum, at a monitor magnification of 410 or 1640×. Individual measurements of otolith and somatic growth during the 10-d trials were compared using least squares regressions.

Results

Development of Secondary Growth Centres

Secondary growth centres in larval winter flounder sagittae initially appeared midway through eye migration (Table 1; Fig. 2B). All of the sagittae from stage 1 and 2 larvae were spherical. Almost one third of stage 3 larvae and half of stage 4 larvae had developed at least one secondary growth centre, first evident as a small bud-like protrusion on the sagitta surface. All postlarvae with completed eye migration (stage 5) had well-developed secondary growth centres, as did all epibenthic juveniles examined ($n > 200$, close-up view in Fig. 3). There was a slight tendency for secondary growth centres to initially develop on the right sagitta (Table 1). Secondary growth centres were present on the right but not the left sagitta for four larvae; only one larva had the opposite pattern.

ANOVAs comparing total length between individuals with and without secondary growth centres were conducted to examine whether development of these features was related to larval size as well as stage of eye migration. No significant difference was found for stage 3 larvae ($F = 3.40$, $p = 0.080$), but stage 4 larvae with secondary growth centres were significantly larger than stage 4 larvae without them ($F = 8.33$, $p = 0.013$). Thus, variation in the timing of formation was in part due to variation in fish size.

Bilateral Asymmetry

The left and right sagittae were virtually identical in appearance prior to eye migration (Fig. 2A). The two otoliths diverged

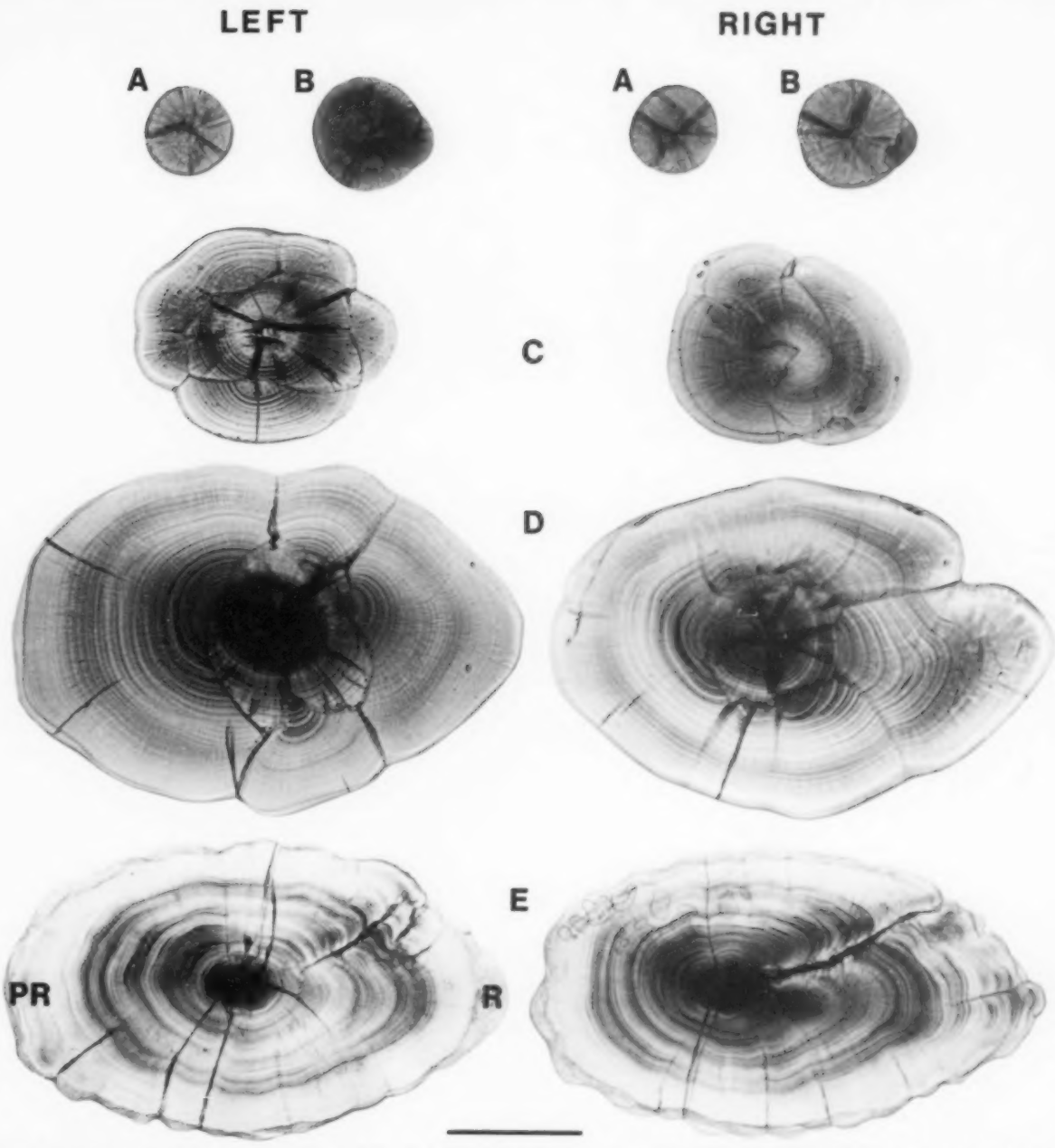

FIG. 2. Left and right sagittae of winter flounder at various stages of development, showing appearance of secondary growth centres and bilateral asymmetry. (A) Stage 2 larva, 6.6 mm SL; (B) stage 4 larva, 7.0 mm SL, with developing secondary growth centres on both the left and right sagitta; (C) recently metamorphosed juvenile, 10.6 mm SL; (D) juvenile, 19.0 mm SL; (E) late juvenile, 63.0 mm SL. Scale bar = 200 μm for Fig. 2A–2D and 525 μm for Fig. 2E. Larval stages are defined in the text. Larval otoliths were whole and photographed in immersion oil; juvenile otoliths were polished on both sides. Otoliths in Fig. 2A–2C show no particular orientation; those in Fig. 2D and 2E are oriented with the anterior edge (rostrum) toward the right and the dorsal edge toward the top. The rostrum (R) and postrostrum (PR) are denoted on the left sagitta in Fig. 2E.

in morphology as growth increased from the secondary growth centres, with greater deposition on the right sagitta toward the anterior, rostral section and greater deposition on the left sagitta toward the posterior, postrostral region (Fig. 2D and 2E). Regressions of radii (left rostral, left postrostral, right rostral, and right postrostral) on standard length indicated highly significant relationships (Fig. 4). Left and right sagittae diverged in their deposition pattern with increasing fish size, as evi-

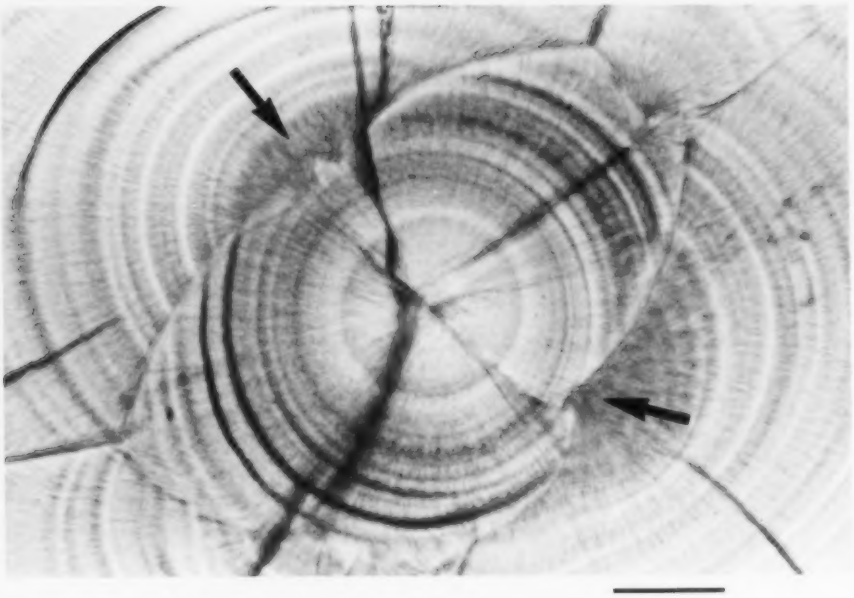

FIG. 3. Photomicrograph of the central region of the left sagitta of a winter flounder (41.5 mm SL). Arrows point to secondary growth centres. Scale bar = 50 μm.

denced by significant differences in the slopes of the regression lines for both the rostrum ($t = 9.57$, $p < 0.001$) and postrostrum ($t = 7.82$, $p < 0.001$).

Although the total length of the sagitta often differed between left and right (Fig. 5), there was no significant tendency for one side to be larger than the other (paired t-test, $t = 0.03$, $p > 0.05$). Paired counts of juvenile increments (those from the innermost secondary growth centre to the otolith edge) on the sagittae also showed no tendency for one side to have more rings than the other (Fig. 5; paired t-test, $t = 1.24$, $p > 0.05$).

Dimensions of lapilli were not quantified, but visual observations suggested that they also show an increasing degree of bilateral asymmetry with increasing fish size. Lapilli were circular in outline and similar on both sides in larvae ($n = 15$), but left and right lapilli of juveniles diverged in morphology, primarily in the longitudinal axis (Fig. 6; $n = 46$). Secondary growth centres did not occur in lapilli.

Daily Increment Validation

Winter flounder juveniles held in cages in the field varied markedly in growth over the 10-d-long experiments. Growth in length during an experiment ranged from −1.5 to +13.3 mm, and growth in weight ranged from −1.3 to +1.6 g. Growth varied depending on the caging location in the estuary and the initial size of the fish when placed in enclosures (Sogard 1990). Flounders starting an experiment at a larger size had slower absolute growth rates than smaller flounders; negative growth occurred only in individuals >50 mm TL at the start of experiments. The wide variability in growth allowed testing of the frequency of increment formation and the relationship between otolith and somatic growth under a variety of environmental conditions that generated growth rates ranging from negative to very fast.

The extent of somatic growth occurring in the 10 d after fish were marked influenced the frequency of detectable increments. The number of increments between the tetracycline mark and the edge of the sagitta matched the number of days elapsed for faster growing fish (Table 2; Fig. 7). Counts were not significantly different from 10 except on the right postrostrum (t-tests). In contrast, sagittae from fish that grew <2.5 mm during the 10-d experiments averaged significantly <10 increments along all four sagittal radii (t-tests, all $p < 0.01$). Within this latter group, counts were variable and unrelated to the amount of somatic growth (all correlation coefficients of increments counts with somatic growth were <0.38 and not significant). Lapilli from the two groups had similar results, with counts underestimated in poorly growing fish ($\bar{x} = 3.9$, SD = 3.1, $n = 7$) but not different from 10 for fish with good growth ($\bar{x} = 9.6$, SD = 0.4, $n = 15$).

Although some material was added to the otoliths of all fishes examined, including those with negative somatic growth, the pattern of otolith deposition seemed to shift in some slowly growing individuals. In these, deposition was not concentrated along the longitudinal (rostral–postrostral) axis (Fig. 7), but was displayed by growth in width rather than length. The lack of material following the mark was not due to grinding and polishing because counts were made on whole otoliths. Little or no deposition occurred in the rostral and postrostral regions, where increment counts were made.

Examination of 23 sagittae with SEM produced equivocal results. The tetracycline-marked increment, determined by matching with UV photomicrographs, was typically deeply etched. Increments on either side of the mark, however, varied greatly in degree of etching. Otoliths of the fast-growing flounders had some well-etched and discernible increments deposited during the caging period, but adjacent increments, visible under light microscopy, could not be identified with SEM. In a few slow-growing juveniles, the tetracycline mark under UV microscopy could be traced in dorsal or ventral sections, but merged with the otolith edge prior to entering the rostrum or

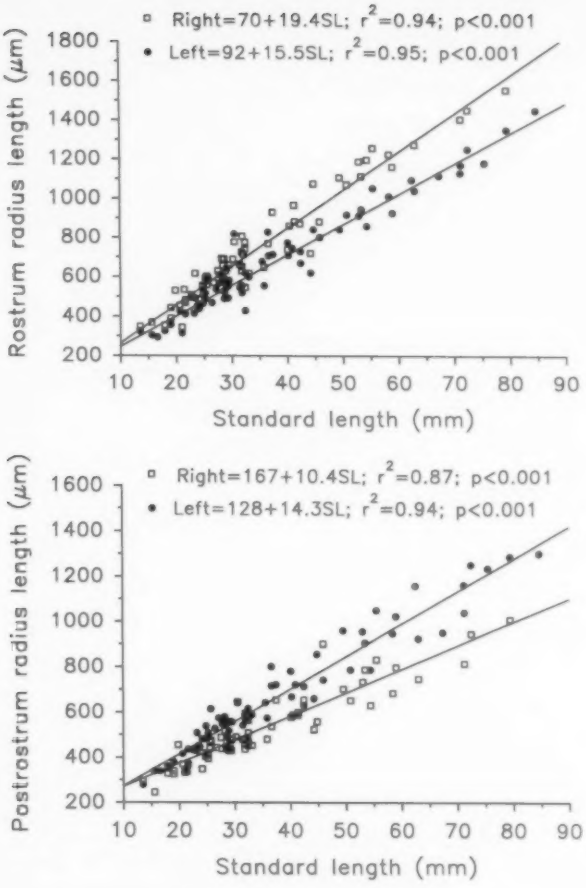

FIG. 4. Relationship of radial lengths of polished sagittae to standard length in juvenile winter flounder. Top = rostral radius; bottom = postrostral radius. Least squares regression lines are plotted and parameters given for left and right radial measurements.

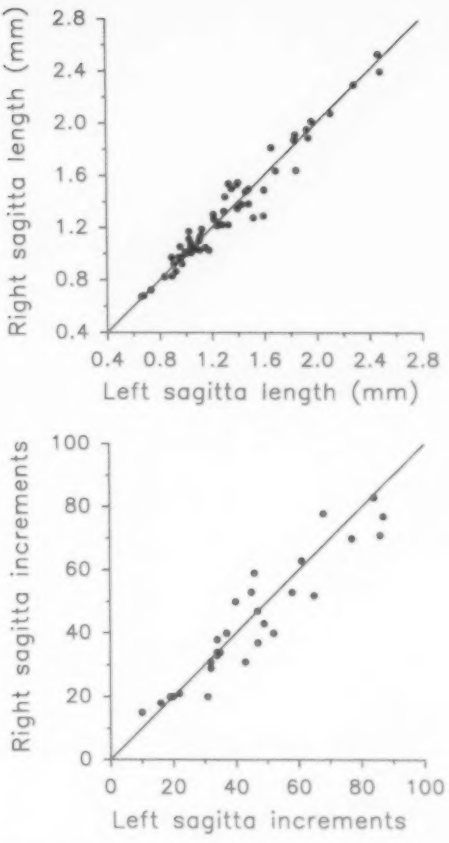

FIG. 5. Lengths (top) of the left and right sagitta from 61 juvenile winter flounder and counts (bottom) of juvenile increments (from the innermost secondary growth centre to the edge) on the left and right sagitta of 29 winter flounder. Increments were counted toward the rostrum of the right sagitta and toward the postrostrum of the left sagitta. Counts represent duration of the postmetamorphic stage, ignoring age of larvae prior to metamorphosis. For both length measurements and increment counts the data do not deviate significantly from the displayed line of one-to-one correspondence.

postrostrum. Tracing the tetracycline mark of these otoliths under SEM examination confirmed the lack of deposition on the longitudinal axis during the caging period. SEM did improve resolution of closely spaced increments not apparent under light microscopy in other slow-growing individuals with at least some rostral or postrostral deposition, but still indicated <10 increments. Resolution problems may have been responsible for some of the failure to consistently detect daily increments.

Otolith and Somatic Growth Relationships

Distances measured from the deeply etched tetracycline mark to the sagitta edge on SEM photographs were consistent with those measured on whole otoliths under light microscopy. Otolith deposition in the outermost portions of the rostrum and postrostrum ranged from 0 to 202 μm over the 10-d experiments.

Otolith growth during caging experiments was significantly related to somatic growth in both length and wet weight (Table 3; Fig. 8). The strength of this correspondence varied according to the radius used in regression analysis, with the left rostrum providing the best correlations (r^2 = 0.73 for growth in length and 0.68 for growth in weight). Initial and final weights of each fish were log-transformed prior to calculating the growth in weight. For somatic growth in length, the lack of evidence of nonlinearity and the homoscedastic distribution of residuals around the linear fit suggested an isometric relationship over the size range of winter flounder examined.

Discussion

Growth of winter flounder otoliths occurs incrementally and at the periphery. Sagittal growth has only one primordium as a focus for growth during the larval period. Initiation of secondary growth foci at the periphery is associated with metamorphosis. Deposition from these rapidly overgrows the nuclear region laid down during the larval period. The physical appearance of the centre region of juvenile winter flounder sagittae (Fig. 3) is remarkably similar to that of starry flounder (Campana 1984a) and European plaice (Karakiri et al. 1989; Alhossaini et al. 1989). Other pleuronectiforms displaying sec-

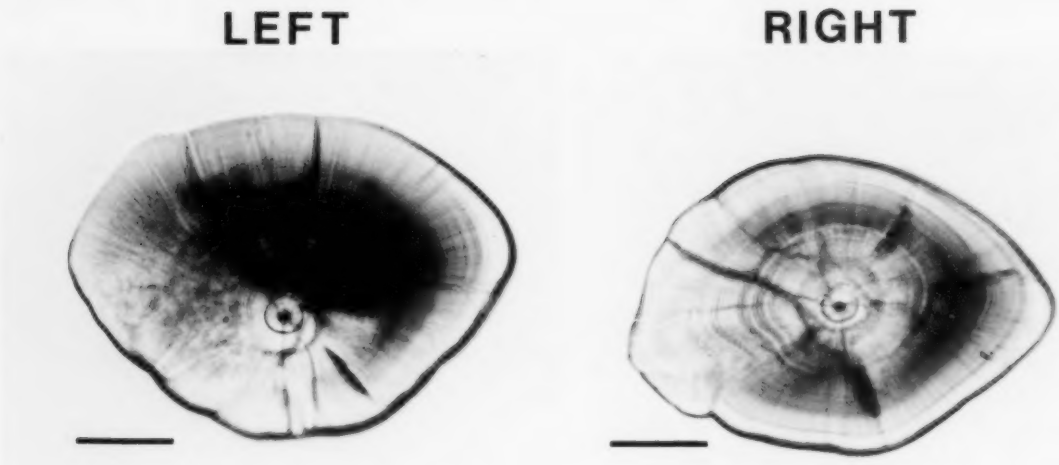

FIG. 6. Left and right lapilli of a juvenile winter flounder (48.1 mm SL). Scale bars = 100 μm. Both lapilli are oriented with the anterior edge toward the right and the dorsal edge toward the top.

TABLE 2. Mean number of otolith increments 10 d after an oxytetracycline mark applied to winter flounder held in field enclosures. Flounders were divided a posteriori into two groups: poor growers grew <2.5 mm during the 10-d experiments and good growers grew >2.5 mm. Counts were made along the rostral and postrostral axis of both the left and right sagitta, but increments along all four radii were not always legible, resulting in the different *n* values.

	Left rostrum	Left postrostrum	Right rostrum	Right postrostrum
Good growers				
Mean	9.63	9.81	10.33	8.71
SD	1.07	1.85	2.54	2.17
n	19	31	18	21
Poor growers				
Mean	4.13	5.33	7.40	4.87
SD	3.04	3.20	2.85	3.14
n	15	15	15	16

ondary growth centres in juvenile sagittae include summer flounder (*Paralichthys dentatus*) (S. Szedlmayer and K. Able, unpubl. data), smallmouth flounder (*Etropus microstomus*) (pers. obs.), and windowpane flounder (*Scophthalmus aquosus*) (pers. obs.). Although the timing of secondary growth centre development in these species has not yet been determined, a common morphological pattern is evident. Secondary growth centres are not, however, unique to flatfish, occurring in many species that maintain normal body orientation as adults (Nishimura and Yamada 1984; Brothers 1984; Campana 1984b). The cause of the abrupt change in the pattern of deposition is presently unknown.

This study provides the first documented evidence of diverging ontogenetic patterns in otolith growth between left and right otoliths, although Smith and Daiber (1977) noted asymmetry in sagittae of adult summer flounder. This bilateral asymmetry implies that the paired otoliths are not functioning as mirror images of each other. The cause of this bilateral variation in deposition is also unknown, but could be due to a mechanical consequence of the 90° shift in body orientation or a functional modification to facilitate hearing and balance control. Graf and Baker (1988) conducted hemilabyrinthectomy operations on adult winter flounder and found that their responses differ from those observed in species that maintain bilateral symmetry. They concluded that the bilateral asymmetry of the labryinth system was necessary for the continued 90° shift in body orientation of flatfish. Thus, the asymmetry of the otoliths is also likely to be related to function. Smith and Daiber (1977) noted greater deposition toward the rostrum of the left sagitta and toward the postrostrum of the right sagitta of summer flounder, a bothid (left-eyed). This pattern is opposite to that found in winter flounder, a pleuronectid (right-eyed). Thus, relative to the substrate the otolith growth pattern is the same for the two species.

The appearance of secondary growth centres provides a valuable time marker of metamorphosis in winter flounder. Although there is some variability in the timing of their formation (Table 1), due in part to larval size, secondary growth centres are present in all winter flounder with completed eye migration. Because eye migration is a rapid process completed in about 1 wk (Chambers and Leggett 1987), estimation of the date of metamorphosis from the date of secondary growth centre appearance is likely to vary within only a few days.

Daily increments have been documented in otoliths of larval winter flounder, but enumeration of the larval increments was imprecise under light microscopy even at an early age, and SEM techniques were required (Radtke and Scherer 1982). The long larval stage duration (approximately 2 mo) (Pearcy 1962; Chambers and Leggett 1987) and small size at metamorphosis (7–9 mm) (Lippson and Moran 1974; Chambers and Leggett 1987) in winter flounder result in slow otolith growth and problems in resolving the narrowly spaced increments in the larval stage. Problematic interpretation of larval stage increments can be bypassed and valuable information obtained on age and

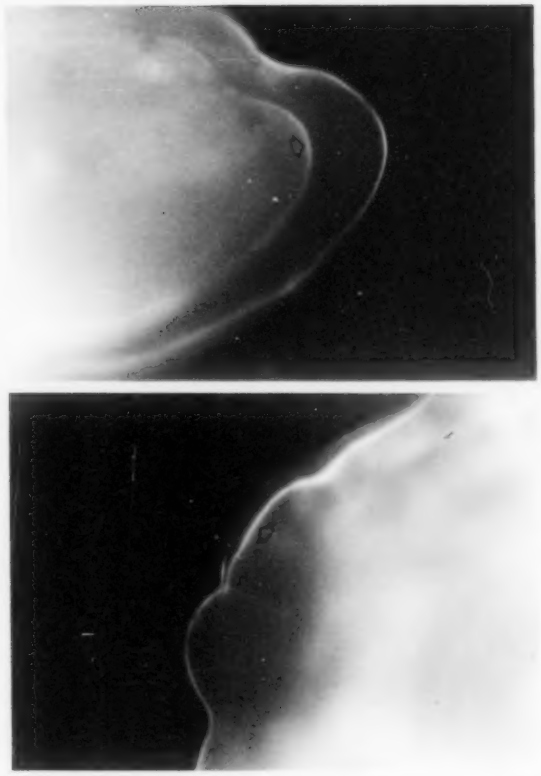

FIG. 7. Sagittae of two juvenile winter flounder with fluorescent marks (arrows) produced by immersion in an oxytetracycline bath; photographed under UV light at 100×. Otolith growth outside the marks occurred over 10 d. The individual in Fig. 7A grew 10.0 mm (TL) during this period whereas the individual in Fig. 7B grew 0.2 mm. The latter otolith demonstrates no growth at the outermost tip of the postrostrum, although growth did occur dorsal to this area (at arrow).

growth patterns from juvenile stage increments deposited outside secondary growth centres.

Several studies have found deposition of daily increments to continue during periods of poor somatic growth or even starvation (Marshall and Parker 1982; Radtke and Dean 1982; Campana 1983; Neilson and Geen 1985; Rice et al. 1985; Eckmann and Rey 1987; Secor et al. 1989). Secor and Dean (1989) outlined a daily increment packing model, in which a minimum amount of material is added daily to the otolith, even if the corresponding somatic growth is negative. Under their model, increments deposited less frequently than daily (Geffen 1982; Lough et al. 1982; McGurk 1984; Siegfried and Weinstein 1989) are attributed to difficulty in resolving narrowly spaced increments. Jones and Brothers (1987) improved the correspondence between increment counts and days elapsed by using SEM, and Campana et al. (1987) concluded that the initial increments deposited in larval herring otoliths were too narrow to be resolved with light microscopy.

Alhossaini and Pitcher (1988), however, obtained results for juvenile European plaice similar to my results for juvenile winter flounder. They also found increments to be less than daily in poorly growing juveniles, and SEM techniques did not elucidate additional increments. Etching problems occurred for both species, preventing discrimination between resolution difficulties and actual less than daily deposition. For a few winter flounder, however, tetracycline marks indicated no growth in the rostrum or postrostrum (Fig. 7), although growth occurred in other parts of the otolith. All of these individuals had extremely low or negative growth during caging experiments, and calcium carbonate deposition did not appear to completely cover the otolith surface, resulting in nondaily increments in the sections where counts were made. For juvenile starry flounder, Campana (1983) also found that increment deposition appeared to cease in some individuals.

In this study, increment formation rates of $<1{\cdot}24\ h^{-1}$ were found primarily in otoliths of slowly growing fish, which were all larger individuals. Based on caging results and length–frequency comparisons of wild-caught fishes, growth rates of winter flounder were very high after settlement from the plankton, but declined rapidly in older juveniles (Sogard 1990). During caging experiments, negative somatic growth and zero deposition along the longitudinal axes of the sagittae occurred only in flounders >50 mm TL and only in certain habitats. This low growth rate may not be encountered in free-ranging flounders, but misinterpretation of increment patterns appears more likely in older juveniles. A means of recognizing declining growth rates in the field, by detection of decreased increment widths or some other measure, would be valuable in suggesting potential interpretation problems.

TABLE 3. Results of otolith growth (μm) regressed on somatic growth in total length (mm) and wet weight (g) for juvenile winter flounder during 10-d caging experiments. Sagittal growth was measured along four radii (left rostral, left postrostral, right rostral, and right postrostral). Initial and final wei weights were log-transformed prior to analysis.

Radius	n	Slope	Intercept	r^2	F	P
Sagittal growth vs. growth in length						
Left rostrum	104	8.71	24.02	0.73	273.4	<0.001
Left postrostrum	115	4.86	44.16	0.46	96.4	<0.001
Right rostrum	103	8.42	46.03	0.55	125.1	<0.001
Right postrostrum	106	5.34	23.05	0.58	143.3	<0.001
Sagittal growth vs. growth in weight						
Left rostrum	104	190.04	25.29	0.68	221.8	<0.001
Left postrostrum	115	104.25	11.38	0.43	83.9	<0.001
Right rostrum	103	187.59	46.89	0.53	114.9	<0.001
Right postrostrum	106	124.48	22.83	0.60	158.9	<0.001

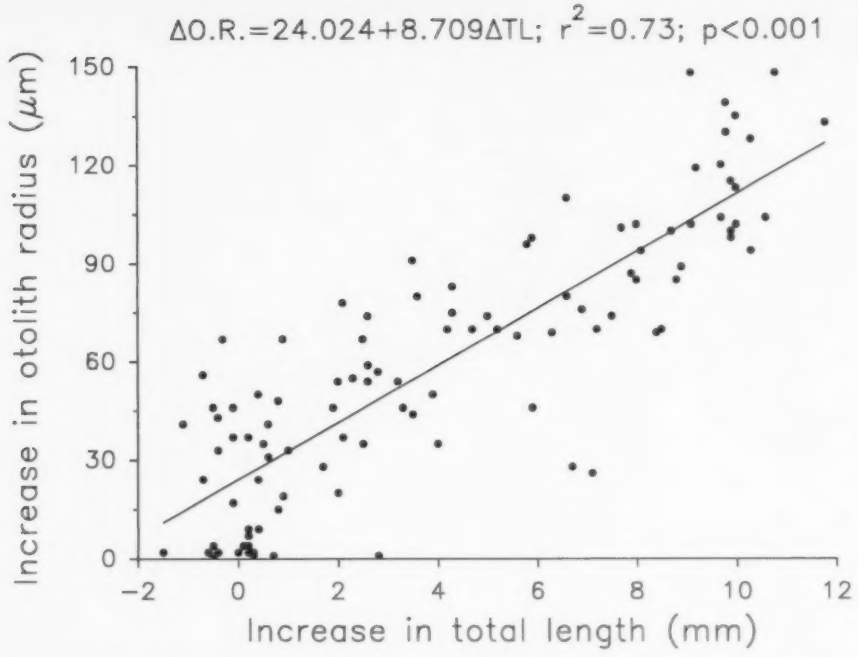

FIG. 8. Relationship of otolith growth to somatic growth (TL) over a 10-d period for juvenile winter flounder held in cages in different habitats in the field (r^2 = 0.73, n = 104). Otolith growth was measured along the rostral radius of the left sagitta. Results of the regression analyses for measurements along all four radii are presented in Table 3.

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References

AHLSTROM, E. H., K. AMAOKA, D. A. HENSLEY, H. G. MOSER, AND B. Y. SUMIDA. 1984. Pleuronectiformes: development, p. 641–670. *In* H. G. Moser [ed.] Ontogeny and systematics of fishes. American Society of Ichthyologists and Herpetologists Special Publication 1. Allen Press, Lawrence, KS.

ALHOSSAINI, M., Q. LIU, AND T. J. PITCHER. 1989. Otolith microstructure indicating growth and mortality among plaice, *Pleuronectes platessa* L., post-larval sub-cohorts. J. Fish Biol. 35 (Suppl. A): 81–90.

ALHOSSAINI, M., AND T. J. PITCHER. 1988. The relation between daily rings, body growth and environmental factors in plaice, *Pleuronectes platessa* L., juvenile otoliths. J. Fish Biol. 33: 409–418.

BRADFORD, M. J., AND G. H. GEEN. 1987. Size and growth of juvenile chinook salmon back-calculated from otolith growth increments, p. 453–462. *In* R. C. Summerfelt [ed.] Age and growth of fish. Iowa State University Press, Ames, IA.

BROTHERS, E. B. 1984. Otolith studies, p. 50–57. *In* H. G. Moser [ed.] Ontogeny and systematics of fishes. American Society of Ichthyologists and Herpetologists Special Publication 1. Allen Press, Lawrence, KS.

CAMPANA, S. E. 1983. Feeding periodicity and the production of daily growth increments in the otoliths of steelhead trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Can. J. Zool. 61: 1591–1597.

1984a. Microstructural growth patterns in the otoliths of larval and juvenile starry flounder, *Platichthys stellatus*. Can. J. Zool. 62: 1507–1512.

1984b. Interactive effects of age and environmental modifiers on the production of daily growth increments in otoliths of plainfin midshipman, *Porichthys notatus*. Fish. Bull. 82: 165–177.

CAMPANA, S. E., J. A. GAGNÉ, AND J. MUNRO. 1987. Otolith microstructure of larval herring (*Clupea harengus*): image or reality? Can. J. Fish. Aquat. Sci. 44: 1922–1929.

CAMPANA, S. E., AND J. D. NEILSON. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42: 1014–1032.

CHAMBERS, R. C., AND W. C. LEGGETT. 1987. Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. Can. J. Fish. Aquat. Sci. 44: 1936–1947.

ECKMANN, R., AND P. REY. 1987. Daily increments on the otoliths of larval and juvenile *Coregonus* spp. and their modification by environmental factors. Hydrobiologia 148: 137–143.

GEFFEN, A. J. 1982. Otolith ring deposition in relation to growth rate in herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. Mar. Biol. 71: 317–326.

GRAF, W., AND R. BAKER. 1988. Hemilabyrinthectomy and selective otolith lesion symptoms in the flatfish. Biol. Bull. 175: 316.

JONES, C., AND E. B. BROTHERS. 1987. Validation of the otolith increment aging technique for striped bass, *Morone saxatilis*, larvae reared under suboptimal feeding conditions. Fish. Bull. 85: 171–178.

KARAKIRI, M., R. BERGHAHN, AND H. VON WESTERNHAGEN. 1989. Growth differences in 0-group plaice *Pleuronectes platessa* as revealed by otolith microstructure analysis. Mar. Ecol. Prog. Ser. 55: 15–22.

LIPPSON, A. J., AND R. L. MORAN. 1974. Manual for identification of early developmental stages of fishes of the Potomac River estuary. Environmental Technical Center, Martin-Marietta, Baltimore, MD. 282 p.

LOUGH, R. G., M. PENNINGTON, G. E. BOLZ, AND A. A. ROSENBERG. 1982. Age and growth of larval Atlantic herring, *Clupea harengus* L., in the Gulf of Maine – Georges Bank region based on otolith growth increments. Fish. Bull. 80: 187–199.

MARSHALL, S. L., AND S. S. PARKER. 1982. Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths. Can. J. Fish. Aquat. Sci. 39: 542–547.

MCGURK, M. D. 1984. Ring deposition in the otoliths of larval Pacific herring *Clupea harengus pallasi*. Fish. Bull. 82: 113–120.

MOSEGAARD, H., H. SVEDANG, AND K. TABERMAN. 1988. Uncoupling between somatic and otolith growth in arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. Can. J. Fish. Aquat. Sci. 45: 1514–1524.

NEILSON, J. D., AND G. H. GEEN. 1985. Effects of feeding regimes and diel temperature cycles on otolith increment formation in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Fish. Bull. 83: 91–101.

NISHIMURA, A., AND J. YAMADA. 1984. Age and growth of larval and juvenile walleye pollock *Theragra chalcogramma* (Pallas), as determined by otolith daily increments. J. Exp. Mar. Biol. Ecol. 82: 191–205.

NOLF, D. 1985. Otolithi piscium, p. 1–26. *In* H. P. Schulze [ed.] Handbook of paleoichthyology. Vol. 10. Gustav Fischer Verlag, New York, NY.

PEARCY, W. G. 1962. Ecology of an estuarine population of winter flounder, *Pseudopleuronectes americanus* (Walbaum). Parts I–IV. Bull. Bingham Oceanogr. Collect. Yale Univ. 18: 1–78.

RADTKE, R. L. 1989. Larval fish age, growth, and body shrinkage: information available from otoliths. Can. J. Fish. Aquat. Sci. 46: 1884–1894.

RADTKE, R. L., AND J. M. DEAN. 1982. Increment formation in the otoliths of embryos, larvae and juveniles of the mummichog, *Fundulus heteroclitus*. Fish. Bull. 80: 201–205.

RADTKE, R. L., AND M. D. SCHERER. 1982. Daily growth of winter flounder (*Pseudopleuronectes americanus*) larvae in the Plymouth Harbor estuary, p. 1–5. *In* C. F. Bryan, J. V. Conner, and F. M. Truesdale [ed.] Fifth annual larval fish conference, Louisiana Cooperative Fishery Research Unit, Louisiana State University, Baton Rouge, LA.

REZNICK, D., E. LINDBECK, AND H. BRYGA. 1989. Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). Can. J. Fish. Aquat. Sci. 46: 108–112.

RICE, J., L. B. CROWDER, AND F. P. BINKOWSKI. 1985. Evaluating otolith analysis for bloater *Coregonus hoyi*: do otoliths ring true? Trans. Am. Fish. Soc. 114: 532–539.

SECOR, D. H., AND J. M. DEAN. 1989. Somatic growth effects on the otolith – fish size relationship in young pond-reared striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 46: 113–121.

SECOR, D. H., J. M. DEAN, AND R. B. BALDEVARONA. 1989. Comparison of otolith growth and somatic growth in larval and juvenile fishes based on otolith length/fish length relationships. Rapp. P.-v. Réun. Cons. int. Explor. Mer 191: 431–438.

SIEGFRIED, R. C. III, AND M. P. WEINSTEIN. 1989. Validation of increment deposition in the otoliths of spot (*Leiostomus xanthurus*). Estuaries 12: 180–185.

SMITH, R. L., AND F. C. DAIBER. 1977. Biology of the summer flounder, *Paralichthys dentatus*, in Delaware Bay. Fish. Bull. 75: 823–830.

SOGARD, S. M. 1990. Parameters of habitat quality for epibenthic fishes and decapod crustaceans in New Jersey estuaries. Ph.D. thesis, Rutgers University, New Brunswick, NJ.

VOLK, E. C., R. C. WISSIMAR, C. A. SIMENSTAD, AND D. M. EGGERS. 1984. Relationship between otolith microstructure and the growth rate of juvenile chum salmon (*Oncorhynchus keta*) under different prey rations. Can. J. Fish. Aquat. Sci. 41: 126–133.

WILSON, K. H., AND P. A. LARKIN. 1982. Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry. Can. J. Fish. Aquat. Sci. 39: 1335–1339.

WRIGHT, P. J., N. B. METCALFE, AND J. E. THORPE. 1990. Otolith growth and somatic growth rates in Atlantic salmon parr, *Salmo salar* L: evidence against coupling. J. Fish Biol. 36: 241–249.

A Method for Estimating Spill Effectiveness for Passing Juvenile Salmon and Its Application at Lower Granite Dam on the Snake River

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Wilson, J. W., A. E. Giorgi, and L. C. Stuehrenberg. 1991. A method for estimating spill effectiveness for passing juvenile salmon and its application at Lower Granite Dam on the Snake River. Can. J. Fish. Aquat. Sci. 48: 1872–1876.

Juvenile salmonids migrating down the Snake and Columbia rivers in the northwestern United States pass each of the rivers' hydroelectric dams via either the powerhouse or the spillway. Spill effectiveness, the proportion of juveniles passing through the dam via the spillway, is important to fishery managers seeking to minimize the exposure of fish to turbines. We outline a method for estimating spill effectiveness and its sampling variance using a multinomial model with categories defined on the basis of information recovered from radio tagged fish. We applied this method to data from tagged chinook salmon (*Oncorhynchus tshawytscha*) juveniles released at Lower Granite Dam in 1985. For two release conditions, one with 20 and the other with 40% of the total water volume going through the spillway, the maximum likelihood estimates of spill effectiveness were 41 and 61%, respectively. Increasing the flow of water through spillways may therefore be a useful method of increasing spill passage of juvenile salmonids at Lower Granite Dam. Similar radio tag experiments and multinomial models could readily be devised to study spill effectiveness at hydroelectric projects with a wide variety of structural plans.

Les saumons juvéniles qui descendent la rivière Snake et le fleuve Columbia dans le nord-ouest des États-Unis franchissent tous les barrages hydro-électriques sur leur cours, soit par la centrale, soit par le déversoir. L'efficacité du passage par le déversoir, ou proportion de juvéniles franchissant le barrage par le déversoir, est un facteur important pour les éleveurs, ceux-ci cherchant à éviter autant que possible que les poissons soient exposés aux dangers des turbines. Nous présentons ici une méthode qui permet d'estimer l'efficacité du passage par les déversoirs ainsi que la variance d'échantillonnage qui s'y associe au moyen d'un modèle multinomial comportant des catégories définies d'après des données recueillis en suivant des poissons porteurs d'un radio-émetteur. Nous avons appliqué cette méthode à des données recueillies sur des saumons quinnat (*Oncorhynchus tshawytscha*) juvéniles lâchés au barrage Lower Granite en 1985. Nous avons considéré deux types de lâchage : l'un où 20 % du volume d'eau total passe par le déversoir, l'autre où la proportion est de 40 %; la probabilité maximale de l'efficacité des déversoirs a été estimée à 41 et 61 % respectivement. L'augmentation du débit des déversoirs semble donc être un moyen pratique d'accroître le passage des salmonidés juvéniles dans le déversoir au barrage de Lower Granite. Il serait facile de concevoir des expériences semblables avec radio-émetteur et modèles multinomiaux pour étudier l'efficacité des déversoirs de divers types d'ouvrages hydro-électriques.

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During the spring and summer, salmonid (*Oncorhynchus* sp.) juveniles (smolts) migrate downstream from tributaries in the Columbia River basin (northwestern United States) toward the Pacific Ocean. Depending on the location of their spawning sites, smolts may pass up to nine large hydroelectric dams on the Columbia River alone during their migration. They may also have to pass numerous smaller dams to reach the Columbia River. The major fish passage conduits at the dams in the Columbia River system are the powerhouse, which contains the turbines used for power generation, and the spillway, where excess water is allowed to flow past the dam without generating power. Generally, the dam operators can allocate river flow between the powerhouse and spillways.

The decline of salmon populations concurrent with the construction of the dams led to studies on the effects of dams on migrant survival. Such studies generally concluded that juvenile fish passing through turbines died at a higher rate than did those passing through spillways (reviewed in Raymond 1979; Ruggles and Murray 1983; Stier and Kynard 1986). Schoeneman et al. (1961) estimated the mean survival for chinook salmon (*Oncorhynchus tshawytscha*) fingerlings passing over the spillway at McNary Dam at 98%. Similar levels of spillway survival were estimated at Bonneville Dam. At that site, Holmes (1952) estimated that on the average, 97% of fingerling chinook salmon released through the spillway survived relative to a downstream control release group. Bell and DeLacy (1972) noted variable survival rates of juvenile salmonids passing over

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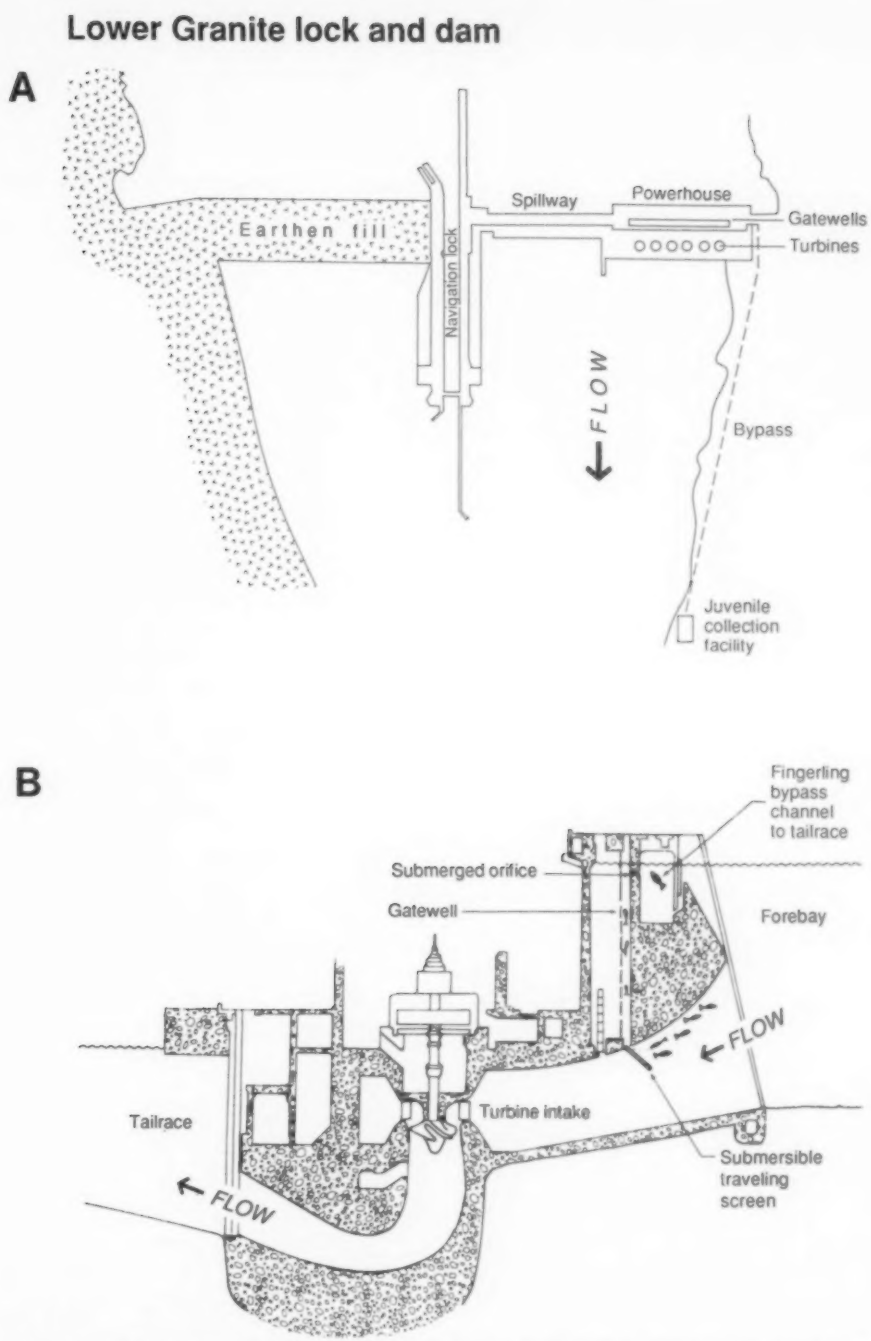

FIG. 1. (A) Overhead view of Lower Granite Lock and Dam on the Snake River; (B) cross-section of a turibne unit in the powerhouse. Antennas for radiotelemetry monitors were deployed in the gatewell and on the face of the dam.

spillways throughout the Pacific Northwest. Ruggles and Murray (1983) in their review of spillway investigations in Canada noted that spillways are generally considered benign passage routes but that at certain sites, mortality was substantial. Turbine survival estimates at different dams vary widely. Schoeneman et al. (1961) estimated survival rates for fingerlings passing through turbines at McNary and Bonneville dams as 89 and 89.5%, respectively. Bell (1981) presented survival estimates from numerous studies conducted in the United States which range from less than 1 to 99%. One result of these studies has

been the practice of increasing the proportion of water flowing through the spillway during smolt outmigration, enabling more fish to pass the dam via that route (Raymond 1979).

The proportion (percent) of the migrant population which passes a hydroelectric project via the spillways is termed "spill effectiveness." Estimates of this proportion are useful in assessing the success of diverting migrating smolts from the turbines. In addition, such estimates are necessary for current modeling efforts directed toward estimating cumulative effects of hydroelectric passage on juvenile salmon (Northwest Power Planning Council 1987). Hydroacoustic methods of fish counting have been used to estimate this quantity (e.g. Kuehl 1986). However, such methods cannot be used to study a particular species in a multispecies population such as that in the Columbia–Snake River system because they cannot directly identify the species being counted. A second drawback of these methods is that they do not offer a means of estimating the sampling variance of the spill effectiveness estimate. In this paper, we present a method of estimating spill effectiveness that can be used to study an individual species and which affords a sampling variance estimate. We applied the method to chinook salmon smolts released upstream from Lower Granite Dam in 1985. We also discuss briefly some management implications of the results.

Methods

Data Collection

Lower Granite Dam spans the Snake River about 173 km upstream from the Snake River's confluence with the Columbia River and about 695 km upstream from the Pacific Ocean (Fig. 1A). Migrating juvenile salmonids may pass the dam via the spillway, the turbines, or a special bypass system installed in the powerhouse. As downstream migrants enter the powerhouse, some are diverted by a submersible traveling screen upward into the gatewell. Fish leave the gatewell and enter the bypass channel, which either delivers fish to the juvenile fish collection facility or returns them to the river downstream from the dam (Fig. 1B).

To monitor juvenile passage routes past the dam, we inserted 30-MHz juvenile radiotelemetry tags, each transmitting a unique pulse-generated code, into large (greater than 150 mm fork length) chinook salmon smolts. We obtained test fish at the juvenile collection facility and held them following tagging in 1.3-m-diameter tanks for a minimum of 10 h prior to release. We then transported the fish to the release site in 132-L plastic containers, lowered the containers to water level, and poured the fish into the river. We released groups of tagged smolts about 4.8 km upstream for the dam, approximately 50% of each group on the spillway side of the river and the remainder on the powerhouse side. We released fish under two water flow regimes, 40% spill ($N = 100$) and 20% spill ($N = 101$).

We placed radio antennas at several locations: on the upstream faces of the spillway intakes and powerhouse intakes, underwater in the bypass system, and at three sites 1.4, 3.2, and 6.1 km downstream from the dam. Monitors connected to the antennas at each location recorded the coded signals as fish passed the site as well as the time of each detection. We assumed on the basis of field tests that all tagged fish in the bypass system or passing downstream monitors were detected. Simulations show that mild departures from this assumption have a negligible effect on spill effectiveness estimates. In addition, we assumed that it was possible for fish to enter the spillway and powerhouse without being detected by their respective intake monitors.

Modeling and Estimation

Using data recovered from the monitors, we assigned each released fish to one of the following eight detection fates: (1) detected passing via the spillway and again downstream, (2) detected passing via the spillway but not downstream, (3) detected passing via the turbine and again downstream, (4) detected passing via the turbine but not downstream, (5) detected both passing into the powerhouse and in the bypass system, (6) detected only in the bypass system, (7) detected downstream but not at the dam, and (8) not detected after release.

Fish entrained in the bypass system were not released into the river but transported to below Bonneville Dam, the last dam in the Columbia River system. Therefore, there are no fates combining bypass system and downstream detections.

We assumed that the probability of experiencing a particular fate was the same for each fish released and that each fish's fate was independent of all others. If $n_1, n_2, \ldots, n_8$ are the numbers of fish observed in each category and $\pi_1, \pi_2, \ldots, \pi_8$ are the probabilities of the fates, then the n_i are multinomially distributed with

$$P(n_1, n_2, \ldots, n_8 \mid N, \pi_1, \pi_2, \ldots, \pi_8) = \binom{N}{n_1, n_2, \ldots, n_8} \prod_{i=1}^{8} \pi_i^{n_i}$$

where $N = \sum_{i=1}^{8} n_i$ is the number of fish released and $\sum_{i=1}^{8} \pi_i = 1$.

We next wrote the probabilities π_i as

$$\begin{aligned}
\pi_1 &= p_d\, p_s\, p_{fs}\, (1 - p_{ls}) \\
\pi_2 &= p_d\, p_s\, p_{fs}\, p_{ls} \\
\pi_3 &= p_d\, (1 - p_s)\, p_{ft}\, (1 - p_g)\, (1 - p_{lt}) \\
\pi_4 &= p_d\, (1 - p_s)\, (1 - p_g)\, p_{ft}\, p_{lt} \\
\pi_5 &= p_d\, (1 - p_s)\, p_g\, p_{ft} \\
\pi_6 &= p_d\, (1 - p_s)\, p_g\, (1 - p_{ft}) \\
\pi_7 &= p_d\, [p_s\, (1 - p_{fs})\, (1 - p_{ls}) \\
&\qquad + (1 - p_s)\, (1 - p_g)\, (1 - p_{ft})\, (1 - p_{lt})] \\
\pi_8 &= 1 - p_d\, [1 - p_s\, p_{ls}\, (1 - p_{fs}) \\
&\qquad - (1 - p_s)\, (1 - p_g)\, p_{lt}\, (1 - p_{ft})]
\end{aligned}$$

where p_d = probability that a fish migrated to the dam with a functional tag, p_s = probability that a fish reaching the dam passed via the spillway (spill effectiveness), p_g = probability that a fish entering the powerhouse was guided into the bypass system, p_{fs} = probability that a fish passing via the spillway was detected by spillway intake monitors, p_{ft} = probability that a fish passing via the powerhouse was detected by powerhouse intake monitors, p_{ls} = probability that a fish was lost to downstream detection after passing via the spillway, and p_{lt} = probability that a fish was lost to downstream detection after passing via the turbines.

We obtained the maximum likelihood estimator (MLE) for $\boldsymbol{p} = (p_d, p_s, \ldots, p_{lt})'$ using the invariance property of maximum likelihood estimation (Mood et al. 1974): we expressed $\boldsymbol{p}$ as a unique function of $\boldsymbol{\pi}$, $= (\pi_1, \pi_2, \ldots, \pi_8)'$, say $g(\boldsymbol{\pi})$. The MLE for $\boldsymbol{p}$ is $g(\hat{\boldsymbol{\pi}})$, where $\hat{\boldsymbol{\pi}} = (\hat{\pi}_1, \hat{\pi}_2, \ldots, \hat{\pi}_8)'$ and n_i/N, the fraction

of fish observed in the ith detection fate, is the MLE for π_i. Then the estimated spill effectiveness is

$$\hat{p}_s = \frac{A}{A + B}$$

where $A = (n_1 + n_2)(n_1 n_5 + n_5 n_7 - n_3 n_6)$ and $B = n_1(n_3 + n_4 + n_5)(n_5 + n_6)$.

We estimated the sampling variance of $\hat{p}_s$ using the delta method (Seber 1982): let $V(\hat{p}_s)$ be the estimated variance of $\hat{p}_s$. Then

$$V(\hat{p}_s) = \boldsymbol{d}' \Sigma \boldsymbol{d}.$$

Σ is the estimated 7×7 variance–covariance matrix of $\boldsymbol{n} = (n_1, n_2, \ldots, n_7)'$. We estimated its elements σ_{ij} by

$$\sigma_{ij} = \begin{cases} N\hat{\pi}_i(1 - \hat{\pi}_i), & i = j \\ -N\hat{\pi}_i\hat{\pi}_j, & i \neq j \end{cases}$$

$$= \begin{cases} n_i\left(1 - \dfrac{n_i}{N}\right), & i = j \\ \dfrac{-n_i n_j}{N}, & i \neq j. \end{cases}$$

$\boldsymbol{d}$ is the vector of partial derivatives $\partial\hat{p}_s/\partial\boldsymbol{n}$.

Wilson (1987) gave further details on estimator derivation and variance estimation for all parameters. A program for calculating parameter estimates and their sampling variances is available from the first author.

Model Assumptions

Our estimation procedure requires the following assumptions, the first two for the validity of the multinomial model and the remainder for the application of the estimates to all fish passing through the dam.

(1) All fish released had the same probability of experiencing a particular fate. This assumption seems reasonable given that the proportion of water flowing through the spillway was held constant throughout the 48-h experiment.

(2) Each tagged fish's fate was independent of the fates of all other tagged fish. This assumption is difficult to verify and would be invalid if fish remained on the side of the river where they were released. We tested the independence of release and dam arrival sites for each spill condition. Because neither test led us to reject the null hypothesis that the proportions of fish passing through the spillway were equal at $\alpha = 0.05$, we assumed that release and arrival sites were independent (20% spill: $\chi^2 = 3.15, p = 0.076$; 40% spill: $\chi^2 = 0.01, p = 0.92$; 1 df for each test).

(3) Fish detections were correctly reported.

(4) There was no spontaneous loss of tag function. We estimated tag failure in the laboratory at 4.4% between 10 and 72 h after tagging. In the field experiments, all fish passed through the detection system well within this period. This rate of failure did not substantially bias spill effectiveness estimates in simulations. We therefore assumed that the effect of tag failure on the field experiment estimates was negligible.

(5) Survival rates of tagged fish were not affected by the tags or the tagging process. Preseason testing under simulated spill and turbine passage conditions failed to detect any difference in survival of tagged and untagged fish.

TABLE 1. Results of 1985 field experiments at Lower Granite Dam. For each experimental spill condition, we list the number of fish released, numbers observed in the first seven detection fates (n_i), the maximum likelihood estimator of spill effectiveness (MLE), and its estimated standard deviation.

Spill condition (%)	Number released	Number observed							MLE	
		n_1	n_2	n_3	n_4	n_5	n_6	n_7	p_s	SD
20	101	21	5	18	8	19	1	7	0.405	0.060
40	100	31	7	12	7	7	1	8	0.606	0.070

(6) The fish released and recovered were representative of all fish passing through the dam. We assume that the large size of the tagged smolts relative to the entire smolt population does not affect spill effectiveness estimates. We also assume that the estimates are not affected by the selection of a particular smolt stock for tagging.

Stuehrenberg et al. (1986) concluded that tagged and untagged fish of similar size exhibited similar levels of swimming stamina. In addition, no apparent difference was found in smolt survival under simulated spillway and turbine passage conditions. However, differences were observed between tagged and untagged fish with respect to buoyancy. Such a difference might bias parameter estimates by affecting the vertical distribution of smolts, their susceptibility to guidance by submersible traveling screens, and hence their detection fates. Simulations under a variety of dam passage conditions showed that the effect of buoyancy on spill effectiveness estimates was negligible (Wilson 1987).

Results and Discussion

For both spill conditions tested, fish appeared to migrate via the spillway in greater proportion than the prevailing proportion of river flow that was spilled (Table 1). Because of the asymptotic normality of maximum likelihood estimators, it is reasonable to test the null hypothesis that spill effectiveness is equal to the prevailing spill level by comparing the following test statistic with the standard normal distribution (Mood et al. 1974):

$$Z = \frac{\hat{p}_s - \mu}{\mathrm{SE}(\hat{p}_s)}$$

where $\hat{p}_s$ is the estimated spill effectiveness, μ is the hypothesized spill effectiveness (equal to the proportion of water spilled), and $\mathrm{SE}(\hat{p}_s)$ is the estimated standard error of the spill effectiveness estimate. In both spill conditions, we rejected the null hypothesis using two-tailed tests (20% spill: $Z = 3.41$, $p = 0.0003$; 40% spill: $Z = 2.80$, $p = 0.003$). These results suggest that fish pass through the spillway at a rate significantly higher than the proportion of water discharged as spill. Thus, at this dam, spill is a particularly efficient means to pass fish.

We believe that our finding may be a consequence of two factors. First, during the experiments reported here, a debris-trapping floating log boom spanned the forebay in front of the powerhouse; this is the normal condition at this site. Smolts swimming near the surface of the water may have been guided along its face until they arrived at the spillway. Second, the powerhouse is close to the southern shore whereas the spillway is close to the middle of the river (Fig. 1). As a result, fish approaching the dam on the northern side of the river must traverse the spillway before encountering the powerhouse.

More generally, unless the distribution of fish is skewed toward the southern shore, the spillway fish proportion should exceed the proportion of water spilled.

A similar result was observed at John Day Dam in 1984, when Giorgi et al. (1985) estimated a spill effectiveness of 74% of the radio tagged yearling chinook salmon passing over the spillway controlling 42% of the total water flow. In addition, they tracked by boat the migration routes of individual smolts swimming through the reservoir. Fish appeared to be shunted to the spillway side of the river by the influx of the John Day River just upstream from the dam. Giorgi et al. (1985) also used purse seines to sample transects across the reservoir and found that fish density was highest on the spillway side of the river. The consistency of the radio tag and purse seine results strengthens our conviction that radio tagging does not substantially alter behavior affecting the horizontal distribution of fish in the river.

Kuehl (1986) estimated spill effectiveness from hydroacoustic estimates of fish passage at Lower Granite Dam at the same time as our radio tag releases. Her estimates were 19 and 35% during the 20 and 40% spill conditions, respectively. Why radio tag and hydroacoustic experiments yielded such different results is not clear, but two factors may have contributed to the difference. First, the radio tag releases yielded information about yearling chinook salmon only whereas the hydroacoustic data reflected all fish migrating in the river. To the extent that chinook salmon smolts migrated differently from other fish, the hydroacoustic and radio tag estimates would have differed. Second, spill effectiveness estimates obtained using hydroacoustics may be highly variable (Kuehl 1986). In addition, it is likely that our sampling variance estimates are unrealistically low because they are based on a probability model that idealizes the migration and radio detection processes. As a result, the differences between the hydroacoustic and radio tag estimates may reflect sampling variability rather than any major difference between the two estimation techniques.

The main purpose of increasing the proportion of water flowing through the spillway is to divert migrating smolts from turbines. From a fisheries management standpoint, it is therefore pertinent to ask whether the proportion of the migrant population passing through the spillway was greater during the 40% spill condition than during the 20% spill condition. We tested the null hypothesis of equal proportions during the two spill conditions using the following test statistic:

$$Z = \frac{\hat{p}_{s,40\%} - \hat{p}_{s,20\%}}{[V(\hat{p}_{s,40\%}) + V(\hat{p}_{s,20\%})]^{1/2}} = 2.175$$

where $\hat{p}_{s,i\%}$ is $\hat{p}_s$ at the $i\%$ spill condition and $V(\hat{p}_{s,i\%})$ is the estimated variance of $\hat{p}_{s,i\%}$. Comparing Z with the standard normal distribution, $P(Z \geq 2.175) \approx 0.015$ and we rejected the null hypothesis in favor of the alternative that $p_{s,40\%} \geq p_{s,20\%}$.

Finally, we note that the strategy of increasing spill passage is used in an effort to increase the survival of migrating fish at hydroelectric projects throughout North America (Bell and DeLacy 1972; Bell 1981). Although our methods were developed in the context of the Columbia–Snake River system, they are directly applicable at any project structurally similar to Lower Granite Dam. Similar radio tag experiments and multinomial models could readily be devised to accommodate a wide variety of structural plans at different projects. The efficacy of passing fish via the spillway could thus be studied using our general approach in other geographic areas as well.

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References

Bell, M. C. 1981. Updated compendium on the success of passage of small fish through turbines. Rep. to U.S. Army Corps of Engineers, Portland, OR. 204 p. (Available from U.S. Army Corps of Engineers, Portland, OR 97208)

Bell, M. C., and A. C. DeLacy. 1972. A compendium on the survival of fish passing through spillways and conduits. Rep. to U.S. Army Corps of Engineers, Portland, OR. 121 p. (Available from U.S. Army Corps of Engineers, Portland, OR 97208)

Giorgi, A. E., L. C. Stuehrenberg, D. R. Miller, and C. W. Sims. 1985. Smolt passage behavior and flow-net relationship to the forebay of John Day Dam. Final Res. Rep. to Bonneville Power Administration, Portland, OR. 68 p. (Available from Northwest Fisheries Science Center, CZES Division, 2725 Montlake Blvd. E., Seattle, WA 98112)

Holmes, H. B. 1952. Loss of salmon fingerlings in passing Bonneville Dam as determined by marking experiments. Rep. to National Marine Fisheries Service, Seattle, WA. (Available from National Marine Fisheries Service Library, 2725 Montlake Blvd. E., Seattle, WA 98112.)

Kuehl, S. 1986. Hydroacoustic evaluation of fish collection efficiency at Lower Granite Dam in spring 1985. Final Rep. to U.S. Army Corps of Engineers, Walla Walla, WA. 106 p. (Available from Northwest Fisheries Science Center, CZES Division, 2725 Montlake Blvd. E., Seattle, WA 98112)

Mood, A. M., F. A. Graybill, and D. C. Boes. 1974. Introduction to the theory of statistics. McGraw-Hill Book Co., New York, NY. 564 p.

Northwest Power Planning Council. 1987. Columbia River Basin fish and wildlife program. Northwest Power Planning Council, Portland, OR 97208. 246 p. (Available from Northwest Power Planning Council, Suite 1100, 850 SW Broadway, Portland, OR 97305)

Raymond, H. L. 1979. Effects of dams and impoundments on migrations of juvenile chinook salmon and steelhead from the Snake River, 1966 to 1975. Trans. Am. Fish. Soc. 108: 505–529.

Ruggles, C. P., and D. G. Murray. 1983. A review of fish response to spillways. Can. Tech. Rep. Fish. Aquat. Sci. 1172: 31 p.

Schoeneman, D. E., R. T. Pressey, and C. O. Junge, Jr. 1961. Mortalities of downstream migrant salmon at McNary Dam. Trans. Am. Fish Soc. 90: 58–72.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters. Griffin and Co., London. 654 p.

Stier, D. J., and B. Kynard. 1986. Use of radio telemetry to determine the mortality of Atlantic salmon smolts passed through a 17-MW Kaplan turbines at a low-head hydroelectric dam. Trans. Am. Fish. Soc. 115: 771–775.

Stuehrenberg, L. C., A. E. Giorgi, C. W. Sims, J. Ramonda-Powell, and J. Wilson. 1986. Juvenile radio-tag study: Lower Granite Dam. Final Res. Rep. to Bonneville Power Administration, Portland, OR. 184 p. (Available from Northwest Fisheries Science Center, CZES Division, 2725 Montlake Blvd. E., Seattle, WA 98112)

Wilson, J. W. 1987. A method of estimating spill effectiveness and fish guidance efficiency for fish passage through hydroelectric dams in the Columbia River Basin. M.S. thesis, University of Washington, Seattle, WA. 44 p. (Available from the University of Washington Library, Seattle, WA 98195)

Efficiency of a Brush Sampler to Measure Periphyton in Streams and Lakes[1]

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Natural periphytic communities are usually sampled by scraping all the algae from a small area. Such a protocol seems more likely to sample loosely attached forms than those which grow tightly attached to the substratum, but this bias has never been quantified. To this end, chlorophyll estimates were obtained by sampling periphyton on cobbles with a brushing syringe sampler in a series of streams and lakes in southern Québec and compared with estimates obtained by immersing the cobble in extractant solvent. In streams, the syringe sampler underestimated chlorophyll concentration, and the severity of this underestimation increased with the current speed. In lakes, the situation was reversed: the brushing syringe gave higher values than those obtained by immersion. These results likely reflect the relative proportions of loosely and tightly attached subcommunities within the periphyton. The latter fraction, which cannot be quantitatively removed by brushing, appears especially important in fast-flowing streams. Ineffective sampling of this fraction may invalidate comparisons obtained with different methods.

Habituellement, pour échantillonner les communautés de périphyton naturelles, on gratte toutes les algues d'une petite superficie. En procédant ainsi, on recueille probablement davantage les formes faiblement fixées que celles qui sont étroitement attachées au substrat; ce facteur d'erreur n'a toutefois jamais été quantifié. Pour ce faire, nous avons comparé les valeurs estimatives de la teneur chlorophyllienne d'échantillons de périphyton prélevés sur des pierres au moyen d'une seringue à brosse dans un ensemble de cours d'eau et de lacs du sud du Québec et obtenus par immersion de pierres dans un solvant. Pour ce qui est des cours d'eau, la concentration de chlorophylle déterminée d'après les prélèvements faits à la seringue a été sous-estimée, l'importance de la sous-estimation augmentant en fonction de la vitesse du courant. Dans le cas des lacs, on a constaté l'inverse : les prélèvements à la seringue à brosse ont donné des valeurs supérieures à celles des échantillons obtenus par immersion dans le solvant. Ces résultats traduisent probablement les proportions relatives de sous-groupes d'organismes du périphyton faiblement ou fortement fixés. Les sous-groupes d'organismes fortement fixés, qui ne peuvent se prélever quantitativement par brossage, semblent particulièrement importants dans les cours d'eau à courant fort. Il se peut que l'inefficacité de l'échantillonnage de cette fraction rende invalide les comparaisons entre les résultats de différentes méthodes.

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The difficulties of quantitative removal of periphyton from its substratum have always hindered the study of this community. Artificial substrata partly circumvent this problem, but their ability to represent natural communities is questionable. Various scraping and brushing techniques can remove periphyton from a known area of the substratum outside the water (Aloi 1990), but when the substrata are too deep or too large to be lifted from the water, the most successful device has been a simple sampler proposed by Stockner and Armstrong (1971). This method uses a brush connected to a syringe that can remove periphyton from a known surface underwater. This technique has proven especially popular since Loeb (1981) modified the design by adding a second syringe to collect the scrapings, thereby improving sampling efficiency. The addition of a neoprene seal to the base of the brushing chamber (Turner et al. 1987) and the provision of an external water supply for rinsing the stone (Flower 1985) were further improvements suggested to reduce contamination of the sample by adjacent loosely attached algae.

In an analysis of 50 papers focusing on periphyton published in the last 5 yr in the leading limnological journals, we found that most studies utilized scraping or brushing to sample chlorophyll concentration on both natural (79%) and artificial substrata (81%). The remaining studies immersed the substrata directly in extracting solvent. When biomass was estimated as biovolume or dry weight, 100% of the studies involved scraping or brushing. Despite the wide acceptance of these techniques, their ability to sample the entire periphytic community has rarely been questioned and never quantified. Unbiased and quantitative sampling is essential both to hypothesis testing and to adequate representation of the community and its ecology. If these bias are large, our knowledge of these communities may be founded on misapprehensions.

In this study, we assess the efficiency of a brushing syringe sampler by comparing chlorophyll values obtained for periphyton removed with Loeb's technique with those derived by direct extraction of the entire substratum. Since biomass and degree

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of attachment of the community could affect the performance of the sampler, we sampled a range of environments with different nutrient levels, current, and periphyton development. If Loeb's sampler is effective, it should give an unbiased estimate of the chlorophyll levels obtained by immersion, regardless of the variation in these environmental conditions.

Methods

All samples were collected within a 100-km radius of Montréal (Québec) during the summer of 1989. In streams, we sampled riffle areas at depths ranging from 15 to 25 cm: a transect was marked by laying a rope numbered at intervals across the stream, and one cobble (5–10 cm in diameter) was selected at each of 8–10 randomly selected numbers. Positions close to the banks were not selected to reduce the effects of extreme currents. In lake littorals, 10–16 cobbles were sampled at 60–70 cm depth within a 10-m radius. At each sampling, half of the cobbles were sampled underwater with a brushing syringe sampler (Loeb 1981) slightly modified by the addition of a neoprene ring around the opening of the syringe to improve the seal with the stone. This device scraped periphyton from an area of 5.7 cm^2 and collected it in a small volume of water. This material was then filtered onto Gelman type A-E glass fibre filters and kept frozen until analysis. The remaining cobbles were simply lifted from the water and put in plastic bags; these samples were refrigerated while in the field and immediately frozen upon arrival in the laboratory (1–2 h after sampling).

For chlorophyll *a* analysis, both the frozen filters and frozen cobbles were immersed in 96% ethanol for 24 h and the extracts read in a spectrophotometer (Bergman and Peters 1980). Additional readings of the extracts at 430 and 410 nm were used as an index of phaeophytin degradation in the samples (% phaeophytin = $138 - 110 \times A_{430}/A_{410}$; A. Cattaneo, unpubl. data), since the ratio of the absorbance at these two wavelengths is correlated with phaeophytin estimates obtained with acidification (Khalanski 1984).

When these initial studies showed that the two methods yielded different results, we tested to determine if a significant fraction of the community was left on the substrata after brushing. On three samplings, we used a nylon nail brush to brush the entire surface of additional cobbles after removal from the water. The material dislodged by the brushing was filtered onto glass fibre filters, while the cobbles with any residual periphyton were directly immersed in ethanol and treated as previously described.

The algae left on the cobbles after brushing were examined with a method similar to that used to observe tightly attached epiphytes on macrophytes (Cattaneo 1978). A "peel off mask" (Shiseido, Tokyo) was applied to the cobble and, when dry, lifted and mounted in Permount (Fisher Scientific, New Jersey).

The surfaces of the cobbles were estimated from their diameters measured along the three main axes (Graham et al. 1988). Since this results in an estimate of the total surface of the cobble, the surface colonized by the periphyton was overestimated. Because the extent of the colonized area is difficult to measure, no correction for this overestimate was attempted. As a result, chlorophyll concentrations based on direct extraction are expressed per unit area of the whole stone. Actual concentrations in the area of colonization will necessarily be higher.

To indicate the range of conditions at the sites sampled, we measured temperature, pH, and total phosphorus (TP) in subsurface samples at each sampling. Phosphorus was analyzed with the ascorbic acid modification of the molybdenum blue technique (Strickland and Parsons 1972) preceded by digestion under pressure with potassium persulfate (Menzel and Corwin 1965). In the streams, the averaged current was measured at three to four positions along the transect where the cobbles were collected with a Pygmy current meter held 10 cm above the stream bottom. Although this technique cannot measure the microflow in close juxtaposition to the periphyton communities, it allows a comparison of the average current regimes at various sites and dates.

Results and Discussion

Since we sampled at different sites and dates (Table 1), we could cover a broad range of environmental conditions, from lakes to fast streams (52 $cm \cdot s^{-1}$), from oligotrophic to eutrophic (TP range 5.5–82.1 $\mu g \cdot L^{-1}$), from slightly acid to alkaline (pH range 6.2–7.9), and a broad range of periphyton development (chlorophyll range 6–122 $mg \cdot m^{-2}$). This range allowed a more general comparison of the relative efficiency of the brushing syringe and the direct extraction methods. The correlation between the two methods was poor ($r^2 = 0.31$, $n = 21$). The direct extraction gave consistently higher results in streams and consistently lower results in lakes (Fig. 1), suggesting an effect of the current on the relative efficiency of the two methods. Indeed there was a significant inverse relationship between the ratio of the chlorophyll estimate obtained with scraping (S) to that from direct extraction (D) and the current (C) (centimetres per second) (Fig. 2):

$$(1)\quad S/D = 0.057 + 7.03/C$$
$$R^2 = 0.84;\ P < 0.0001;\ n = 16.$$

The relationship was not influenced by the biomass on the cobbles, the pH, or the surface area of the stones. It may therefore be used as a tentative correction for the effect of current speed when using the brushing syringe.

The underestimation by the brushing syringe in fast currents may reflect three mechanisms: (1) some algae dislodged by the brush may be swept away by the current, despite the neoprene seal, (2) the algal community may be better developed on the side of the cobble while the syringe-brush sampled only the top, or (3) at faster currents, tightly attached forms, which are increasingly difficult to dislodge with brushing, may dominate the community.

We were able to indicate the more likely of these mechanisms by comparing the amount removed by a nail brush with that left on the stone after this brushing (Table 2). Loss to the flowing water was irrelevant to that comparison because the cobbles were brushed after removal from the river so all algae removed by brushing could be collected. Brushing the entire surface also eliminated the effect of uneven growth across the cobble. Nevertheless, substantial amounts of chlorophyll were left on the stone. These results suggest that the brushing syringe had difficulty removing the tightly attached community in streams. In fast currents, periphyton may tend to grow prostrate on the rock or in crevices where scouring by the stream is minimized (Keithan and Lowe 1985) and where the brush sampler is less effective.

Visual examination of diatoms remaining on the cobbles after nail brushing confirmed this hypothesis. Nondiatom algae could not be identified due to the previous extraction with ethanol. The dominant diatoms were *Achnanthes minutissima*, *Eunotia*,

TABLE 1. Chemical and physical characteristics of the sites and chlorophyll concentrations observed on cobbles using the brushing syringe sampler (S) or direct extraction (D). Only one sample for pH and TP was collected on each date at each site. Values are averages ± standard error. For streams, $n = 4$; for lakes, n = 6–8.

	Date	pH	TP ($\mu g \cdot L^{-1}$)	Current ($cm \cdot s^{-1}$)	Chlorophyll ($mg \cdot m^{-2}$) S	Chlorophyll ($mg \cdot m^{-2}$) D
Streams						
Outlet Cromwell	June 27	6.2	13.2	26	5.40 ± 1.04	16.50 ± 4.73
				17	3.21 ± 0.84	15.72 ± 1.07
	Aug. 3	6.7	10.1	12	11.24 ± 1.72	12.86 ± 1.54
Outlet Echo	June 28	7.7	10.6	44	6.18 ± 0.37	24.24 ± 2.73
				29	6.49 ± 1.41	29.28 ± 6.74
	Aug. 3	7.9	8.8	5	97.79 ± 20.25	69.94 ± 15.27
Inflow Achigan	June 28	7.4	32.4	33	4.83 ± 0.70	12.02 ± 2.78
				23	4.34 ± 1.08	14.96 ± 2.56
	Aug. 3	7.4	31.3	24	3.78 ± 1.60	19.27 ± 6.65
Inflow Connelly	June 27	7.5	20.3	52	1.46 ± 0.84	6.21 ± 0.76
				40	1.16 ± 0.27	11.30 ± 4.16
	Aug. 3	7.1	5.5	24	2.40 ± 0.53	8.12 ± 0.41
Outlet Waterloo	July 6	7.9	82.1	14	33.06 ± 9.37	42.62 ± 5.71
				47	32.38 ± 10.3	122.91 ± 2.03
Inflow Brome	July 6	7.4	24.2	16	11.91 ± 5.09	22.47 ± 1.17
				26	6.78 ± 0.75	16.88 ± 1.55
Lakes[a]						
Cromwell	Aug. 3	6.6	11.5	—	23.61 ± 4.43	16.07 ± 1.86
Croche	Aug. 3	6.9	9.9	—	11.98 ± 3.32	9.65 ± 2.78
Pin Rouge	Aug. 25	7.3	10.7	—	35.84 ± 5.63	16.88 ± 3.16
Achigan	Aug. 25	6.7	9.5	—	30.96 ± 8.18	18.04 ± 1.79
Echo	Aug. 25	7.8	9.6	—	52.37 ± 9.66	31.96 ± 4.54

[a]Physicochemical characteristics from Lafond et al. (1990).

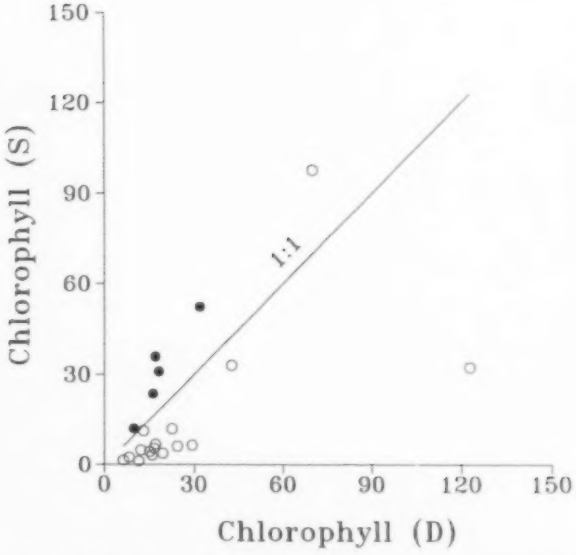

FIG. 1. Chlorophyll values of periphyton communities on cobbles obtained from the brushing syringe method (S) and from direct extraction (D) in lakes (closed symbols) and streams (open symbols).

and *Cocconeis* which covered the surface of the brushed cobbles. All of these are adnate species common to the periphyton on plants and on rocks. This does not appear to be a dying community, for the proportion of phaeophytin was similar in both the fraction removed by brushing and that which remains attached to the stone (Table 2).

In lakes, syringes seem more efficient than direct extraction. This difference between lakes and rivers may be related to the presence of a very loosely held algal fraction in lake communities that is often lost when unenclosed samples are lifted from the water (Gons and van Keulen 1983). It is also possible that samples were enriched by nearby loosely attached algae drawn into the syringe during suction, and this could artefactually increase estimates from the brushing syringe (Flower 1985; Turner et al. 1987). A large part of the discrepancy is also attributable to the different way of calculating the surface area that was sampled by the two methods. The brushing syringe sampler scoured a known area, but the surface area associated with chlorophyll concentrations from direct extraction was the entire surface of the stone, even though only a part of the surface is available for algal colonization. In a study of periphyton on cobbles in a series of New Zealand rivers, Biggs and Close (1989) found that, on average, only 64% of the surface of the cobbles was colonized by the periphyton. If a similar correction is applied to our data, the average overestimation by the brushing syringe in lakes is reduced to 5% and disappears in two of the five cases. On the other hand, such a correction would aggravate the methodological differences observed in streams where direct extraction estimates consistently exceeded those made with the brushing syringe.

Losses during lifting of the stones, overestimation of the colonized area, and enrichment by nearby algae are three potential sources of error that could decrease the estimated periphyton

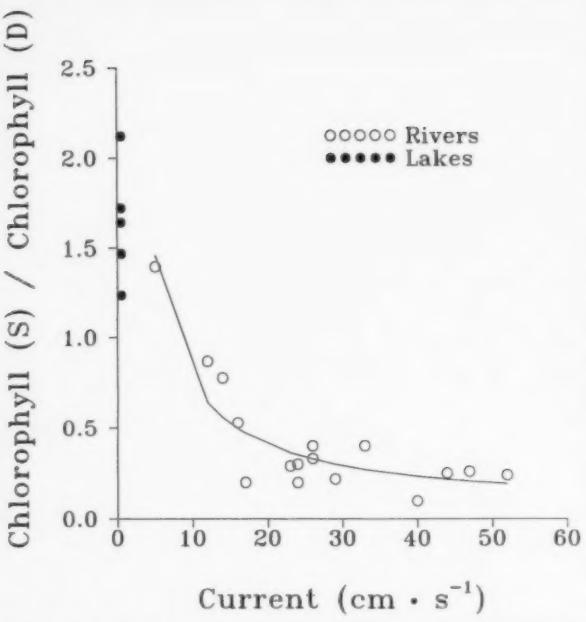

FIG. 2. Relationship between current and the ratio of the chlorophyll values obtained by the brushing syringe method (S) to those obtained by direct extraction (D). The line represents the fit of the equation calculated using the stream data. The values observed in lakes (closed symbols) are plotted at an arbitrary current of zero, although these values were not used in the regression (equation 1).

cover based on direct extraction and increase estimates based on the brushing syringe. Thus the discrepancy between the methods in streams is likely underestimated by equation 1.

Various authors have proposed that periphyton consists of two layers: one loosely held and the other tightly attached (Cattaneo and Kalff 1978; Burkholder and Wetzel 1989). This distinction has been mainly operational, for it is based on the application of different sampling techniques for the two classes. The distinction also has conceptual merit, since the two layers differ taxonomically and are differentially accessible to grazers (Hill and Knight 1987). Our study implies that tightly attached algae are increasingly important in faster current. This community could be especially important in recovery after the scouring of floods and may impose a distinctive character on succession in such sites.

Most periphyton workers are aware of the difficulty of detaching the entire periphyton community from the substratum in fast water, but this suspicion has only rarely been acknowledged. During a study of DMSO extraction efficiency for chlorophyll and ATP, Palumbo et al. (1987) observed that periphyton chlorophyll estimated by scraping river stones with a scalpel was less than half that measured by direct extraction. Jones (1974) found that algal counts of periphyton scraped from river stones were only 9–27% of direct counts of algae on the stone surface, but he attributed the discrepancy to cell damage by the scraping rather than to incomplete removal of the algae. To our knowledge, the present study is the first to quantify the efficiency of different sampling methods over a broad set of conditions. It shows that brushing, either underwater with a brushing syringe or with a nail brush after removal from the water, ignores a significant portion of the community. At currents of approximately 40 $cm \cdot s^{-1}$, the estimates obtained with the brushing syringe were only one quarter of those obtained by direct extraction. This difference applied to both the poorly colonized cobbles of Connelly inflow and the rich growth of Waterloo outlet (Table 1). Since the chlorophyll left on the stone is not degraded, it should represent living algae that could significantly contribute to production and recolonization; however, this fraction would probably be less accessible to grazers in view of its resistance to detachment. Underestimation of periphyton biomass by scraping or brushing techniques is likely to be less severe on artificial substrata such as glass slides and tiles where removal is facilitated by the smooth surface.

At least in streams, most periphyton variables should be measured by direct extraction (Palumbo et al. 1987) from the entire substratum or, if necessary, from fragments chipped off large substrata (Stock and Ward 1989). Cell volume of the tightly attached fraction could be studied after removal with a "peeling off" technique (Margalef 1949; Cattaneo 1978), direct observation with incident light fluorescence microscopy (Jones 1974), or scanning electron microscopy (Hudon and Legendre 1987).

When sampling periphyton, we should be aware of the limits of our techniques. The bias introduced by scraping would differ depending on the environment (lake or stream), the type of substratum, and the goal of the research, but in any case should be taken into account. The discrepancy between the efficiency of the different techniques is especially critical when comparing results from different studies, sites, and substrata. The bias identified here would also be critical in comparing different variables, e.g. chlorophyll concentrations obtained by direct extraction and cell volume and dry weight estimated by scraping. The interpretation of such data could be seriously flawed.

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TABLE 2. Chlorophyll and ratio of absorbances at 430 and 410 nm (430:410) for algae removed by nail brushing and those left on stones. Values in parentheses are the percentage of phaeophytin these ratios represent. Values represent averages ± standard error where $n = 5$. All samples were collected on August 3.

	Removed		Left on stone	
Site	Chlorophyll	430:410	Chlorophyll	430:410
Inflow Connelly	1.74 ± 0.41	1.09 ± 0.02 (18)	5.58 ± 0.72	1.05 ± 0.04 (22)
Inflow Achigan	3.27 ± 0.82	1.19 ± 0.02 (7)	7.45 ± 0.93	1.12 ± 0.05 (15)
Outlet Echo	39.40 ± 3.85	1.18 ± 0.01 (8)	26.44 ± 7.54	1.22 ± 0.01 (4)

References

ALOI, J. E. 1990. A critical review of recent freshwater periphyton field methods. Can. J. Fish. Aquat. Sci. 47: 656–670.

BERGMAN, M., AND R. H. PETERS. 1980. A simple reflectance method for the measurements of particulate pigment in lake water and its application to phosphorus–chlorophyll–seston relationships. Can. J. Fish. Aquat. Sci. 37: 111–114.

BIGGS, B. J. F., AND M. E. CLOSE. 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshwater Biol. 22: 209–231.

BURKHOLDER, J. N., AND R. G. WETZEL. 1989. Microbial colonization on natural and artificial macrophytes in a phosphorus-limited, hardwater lake. J. Phycol. 25: 55–65.

CATTANEO, A. 1978. The microdistribution of epiphytes on the leaves of natural and artificial macrophytes. Br. Phycol. J. 13: 183–188.

CATTANEO, A., AND J. KALFF. 1978. Seasonal changes in the epiphyte community of natural and artificial macrophytes in Lake Memphremagog (Que-Vt). Hydrobiologia 60: 135–144.

FLOWER, R. J. 1985. An improved epilithon sampler and its evaluation in two acid lakes. Br. Phycol. J. 20: 109–115.

GONS, H. J., AND R. VAN KEULEN. 1983. Seasonal changes in organic matter and dark oxygen uptake of epiphyton and epipelon in relation to seston deposition in Lake Vechten (The Netherlands), p. 175–183. *In* R. G. Wetzel [ed.] Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands.

GRAHAM, A. A., D. J. MCCAUGHAN, AND F. S. MCKEE. 1988. Measurement of surface area of stones. Hydrobiologia 157: 85–87.

HILL, W. R., AND A. W. KNIGHT. 1987. Experimental analysis of the grazing interaction between a mayfly and stream algae. Ecology 68: 1955–1965.

HUDON, C., AND P. LEGENDRE. 1987. The ecological implication of growth forms in epibenthic diatoms. J. Phycol. 23: 434–441.

JONES, J. G. 1974. A method for observation and enumeration of epilithic algae directly on the surface of stones. Oecologia 16: 1–8.

KEITHAN, E. D., AND R. LOWE. Primary productivity and spatial structure of phytolitic growth in streams in the Great Smoky Mountains National Park, Tennessee. Hydrobiologia 123: 59–67.

KHALANSKI, M. 1984. Evaluation de la biomasse phytoplanctonique des eaux courantes par le dosage de chlorophylle. Verh. Int. Ver. Limnol. 22: 2010–2019.

LAFOND, M., B. PINEL-ALLOUL, AND P. ROSS. 1990. Biomass and photosynthesis of size-fractionated phytoplankton in Canadian Shield lakes. Hydrobiologia 196: 25–38.

LOEB, S. 1981. An in situ method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. Limnol. Oceanogr. 26: 394–399.

MARGALEF, R. 1949. A new limnological method for the investigation of thin layered epilithic communities. Hydrobiologia 1: 215–216.

MENZEL, D. W., AND H. CORWIN. 1965. The measurement of total phosphorus based on the liberation of organically bound fractions by persulfate oxidation. Limnol. Oceanogr. 10: 280–282.

PALUMBO, A. V., P. J. MULHOLLAND, AND J. W. ELWOOD. 1987. Extraction with DMSO to simultaneously measure periphyton photosynthesis, chlorophyll, and ATP. Limnol. Oceanogr. 32: 464–471.

STOCK, M. S., AND A. K. WARD. 1989. Establishment of a bedrock epilithic community in a small stream: microbial (algal and bacterial) metabolism and physical structure. Can. J. Fish. Aquat. Sci. 46: 1874–1883.

STOCKNER, J. G., AND F. A. J. ARMSTRONG. 1971. Periphyton of the experimental lakes area, northwestern Ontario. J. Fish. Res. Board Can. 28: 215–229.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. 2nd ed. Bull. Fish. Res. Board Can. 167: 310 p.

TURNER, M. A., M. B. JACKSON, D. L. FINDLAY, R. W. GRAHAM, E. R. DE BRUYN, AND E. M. VANDERMEER. 1987. Early response of periphyton to experimental lake acidifications. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 135–149.

Biases Associated with Four Stream Substrate Samplers

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We compared samples collected from 10 substrates of various compositions with a single-probe freeze-core sampler, a triple-probe freeze-core sampler, a McNeil sampler, and a shovel. The accuracy with which these devices sampled particles larger than 50 mm in diameter varied; they were oversampled by the freeze-core devices, sampled in proportion to their availability by a shovel, and sampled inconsistently by the McNeil sampler. The geometric mean particle size and variance of single-probe freeze-core samples consistently exceeded those of samples collected with the other devices. Most sample means also exceeded the test substrate means. By excluding the proportions of particles larger than 50 mm in diameter in our analyses, we found that proportions of several particles sizes in samples collected by different methods differed significantly from the actual proportions in test substrates. There were few differences between the single- and triple-probe freeze-core samples or between McNeil and shovel samples. All four samplers were biased, but the McNeil sampler most frequently produced samples that approximated the true substrate composition.

Nous avons comparé des échantillons prélevés dans dix substrats de composition variable au moyen d'un échantillonneur à carottes (freeze core) à sonde unique, d'un échantillonneur à carottes (freeze core) à sonde triple, d'un échantillonneur McNeil et d'une pelle. La précision du prélèvement d'une particule ayant un diamètre supérieur à 50 mm a varié selon les dispositifs utilisés; ces particules étaient sur-représentées dans les prélèvements faits avec les carrotteurs, étaient échantillonnés en proportion de leur disponibilité avec la pelle, et étaient prélevés de manière inconsistante avec l'échantillonneur McNeil. La taille moyenne géométrique des particules contenues dans les échantillons du carotteur à sonde unique, ainsi que la variance, étaient constamment supérieures à celles des échantillons prélevés au moyen des autres dispositifs. La plupart des moyennes mesurées dans les échantillons dépassaient les moyennes mesurées dans le substrat d'essai. En excluant de nos analyses les proportions de particules supérieures à 50 mm de diamètre, nous avons constaté que les proportions de particules échantillonnées par différentes méthodes et qui appartenaient à différentes classes granulométriques, différaient de manière significative des proportions réelles de ces particules dans les substrats d'essai. Il y a avait peu de différences entre les échantillons prélevés avec l'échantillonneur à sonde simple et les échantillons prélevés avec l'échantillonneur à sonde triple, ou entre les échantillons prélevés avec la sonde McNeil et les échantillons prélevés à la pelle. Les quatre moyens d'échantillonnage comportaient une erreur systématique, mais l'échantillonneur McNeil est celui qui donnait le plus fréquemment des échantillons qui s'apparentaient assez bien à la composition réelle du substrat.

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Fisheries biologists frequently sample substrate composition in streams to assess quality for spawning by salmonids (Stowell et al. 1983) or to detect changes in composition caused by land management (Scrivener and Brownlee 1989). Usually, one of three sampling devices is used to obtain substrate samples: single-probe freeze-core samplers (Walkotten 1976), triple-probe freeze-core samplers (Everest et al. 1980), or McNeil samplers (McNeil and Ahnell 1960). Variations of all these samplers have been developed (Koski 1966; Ringler and Hall 1988). Shovels have also been used to collect substrate samples (P. Carling, Freshwater Biological Association, Far Sawrey, Ambleside, Cumbria, United Kingdom, pers. comm.). Freeze-core samplers freeze interstitial water and nearby substrate to probes that have been driven into the stream bottom; the probes are then extracted with the sample attached. McNeil samplers are forced into the substrate and the material is collected by scooping it onto a rim inside the sampler. Shovels simply skim through the upper layers of the stream bottom. The insertion of all these devices disturbs the substrate before the sample is extracted.

Although each technique samples the substrate in a different way, these differences have been assumed to have little effect on the composition of the sample (Shirazi and Seim 1979). Our objectives in this study were to (1) determine whether samples that were collected from identical substrates by different techniques would have identical compositions, (2) determine whether samples that were collected from test substrates would

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duplicate the composition of those substrates, (3) assess the variation in sample composition associated with each sampler, and (4) identify the particle sizes that were undersampled or oversampled by each technique.

Methods

All tests were conducted at the Sediment Laboratory of the University of Wyoming, Department of Range Management, from 22 March to 18 April 1989. We compared samples collected with four devices: a single-probe freeze-core sampler, a triple-probe freeze-core sampler, a McNeil sampler, and a shovel.

We designed 10 test substrates for these experiments (Table 1). Substrates A–G were designed to reveal biases of the samplers for large or small particles. Substrates H–J simulated redds of different species: H, brook trout (*Salvelinus fontinalis*) (M. K. Young, unpubl. data); I, Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) (Young 1989); and J, coho salmon (*O. kisutch*) (K. V. Koski, National Marine Fisheries Service, Auke Bay, Alaska, unpubl. data). We took a single sample from each of eight replicates of substrates A–D and 16 replicates of E–J (total 128 samples). We collected four samples from test substrates A and C with the single-probe and triple-probe freeze-core samplers, four samples each from test substrates B and D with the McNeil sampler and shovel, and four samples with each device from the remaining six test substrates (E–J). Thus, for comparisons among the samples collected with all devices, we used only substrates E–J. For comparisons between the test substrates and the samples collected with the single-probe freeze-core device and those taken with the triple-probe device, we used substrates A, C, and E–J. Finally, for comparisons between the test substrates and samples collected with the McNeil sampler and those collected with a shovel, we used substrates B, D, and E–J.

Material was sorted on a mechanical shaker through sieves of 10 mesh sizes (millimetres): 50, 25, 12.5, 9.5, 6.3, 3.35, 1.70, 0.85, 0.425, and 0.212; smaller particles were collected on a pan attached to the last sieve. Replicates of each substrate were made individually. The specified proportions (by weight) of each size class were mixed in a large rotating bin for 15 s. The mixture was poured into a sampling container (plastic bucket or tray) that was later filled with enough water to submerge the mixture at least 3 cm. Temperature was held at 18.5°C.

Because each device collects substrate differently, we used separate sampling techniques that allowed all particle sizes to be available to each device. Substrates for both freeze-core samplers and the McNeil sampler were placed in square plastic buckets (20 by 20 by 35 cm). Substrates for the McNeil sampler weighed 12.7 kg and those for the freeze-core samplers weighed 20.0 kg to accommodate the tendency of freeze-core samplers to collect long, relatively narrow samples (Carling and Reader 1981) and of McNeil samplers to collect shorter samples.

To obtain freeze-core samples, we drove either one or three steel probes, 50 cm long and 2 cm in diameter, to the bottom of the plastic bucket containing the test substrate. A steel template held the three probes of the triple-probe freeze-core sampler in an equilateral triangle with 5-cm sides (Everest et al. 1980). We froze the sample to the probe(s) by injecting liquid carbon dioxide into each probe for 2 min. We thawed each sample in a separate plastic bucket.

The diameter and length of the McNeil sampler tube were both 15 cm. The sampler was twisted 15 cm deep into the substrate and all material inside the tube was placed by hand onto a retaining rim. The sampler was then lifted from the substrate, and the material on the rim was washed into a separate plastic bucket.

Test substrates for shovels weighed 10 kg and were placed in rectangular plastic trays (25 by 38 by 13 cm). We inserted a pointed shovel, 21 by 28 cm, straight down into the substrate and then lifted the sample from the tray. After allowing the sample to drain for 2–3 s, we transferred it to a plastic bucket.

All samples were dried in an electric dryer at 60°C for at least 72 h. Next, we sieved the samples as previously described and weighed the amount retained on each sieve on an electronic balance. We described the composition of the samples by calculating the proportion of material retained on each sieve and by calculating the geometric mean particle size by the method of moments (Lotspeich and Everest 1981).

Some devices may not accurately sample large particles (Adams and Beschta 1980; Lotspeich and Reid 1980; Chapman et al. 1986). This inaccuracy may create problems because the presence or absence of such particles can greatly affect the relative proportions of the other size classes. To test for inconsistencies in the sampling of large particles, we regressed the proportion of particles larger than 50 mm in diameter in samples collected by each of the different devices with the proportion of that size in test substrates. We decided that if proportions of this particle size in samples collected by any device were

TABLE 1. Percentages of each substrate size class in test substrates. Type is the identifying label for each test substrate.

	Sieve size (mm)										
Type	50	25	12.5	9.5	6.3	3.35	1.70	0.85	0.42	0.21	0.0
A	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1
B	9.1	9.1	9.1	6.1	12.2	6.7	13.4	12.5	6.6	9.3	6.0
C	16.7	15.2	13.6	12.1	10.6	9.1	7.6	6.1	4.5	3.0	1.5
D	16.7	15.2	13.6	8.1	14.7	6.7	11.2	7.4	2.7	2.2	1.4
E	1.5	3.0	4.5	4.1	9.7	6.7	15.5	17.6	10.3	16.4	10.7
F	9.1	9.1	9.1	6.1	12.2	6.7	20.0	14.8	5.6	4.5	2.9
G	3.0	6.1	9.1	8.1	19.2	6.7	13.4	12.5	6.6	9.3	6.0
H	0.0	0.0	27.7	5.3	11.8	12.2	22.9	14.6	3.3	1.4	0.8
I	10.8	24.8	19.7	4.5	10.3	8.0	12.6	6.1	1.6	1.0	0.6
J	5.2	24.6	19.9	3.3	9.6	5.9	8.4	5.1	3.1	9.0	5.8

biased, i.e. if the slope of the regression was significantly different from unity, or if the proportions were not related to those in the test substrates, i.e. if the regression was not significant, we would recalculate the proportions of the other particle sizes without this size fraction before proceeding with further analyses.

To assess the significance of differences in composition between substrate samples and test substrates, we used the Wilcoxon signed-rank test (Sokal and Rohlf 1981) to compare the proportions of each particle size. Because the proportions of different particle sizes in a single substrate were not independent (thus possibly violating a statistical assumption), we chose the conservative Bonferroni procedure (Neter et al. 1985) to produce an experiment-wide alpha of 0.1 (alpha for individual comparisons = 0.01) for all nonparametric tests comparing samples with test substrates.

To assess the significance of differences among the proportions of each particle size for samples only, we used Kruskal–Wallis two-way analysis of variance (Pimentel and Smith 1990). When this test yielded significant results, we evaluated the significance of differences between samples collected by different pairs of devices by conducting a nonparametric Tukey test (Pimentel and Smith 1990). We selected this test instead of the Bonferroni procedure because we wished to increase the power of the comparisons even though we also increased the likelihood of incorrectly rejecting the null hypothesis. For this and all remaining tests, we considered $P \leq 0.05$ as indicating significance.

We further assessed differences in composition between samples and test substrates by comparing their geometric mean particle sizes. We calculated arithmetic means and standard errors of the geometric mean particle sizes before conducting planned comparisons using *t*-tests (Sokal and Rohlf 1981). In contrast with our analysis of separate particle sizes, we retained particles greater than 50 mm in diameter for the calculation of the geometric mean particle size to demonstrate the effects of ignoring biases associated with large particles. Finally, we regressed the geometric mean particle sizes of the samples collected by different devices on the geometric mean particle sizes of the test substrates to evaluate the variability in estimates of this parameter associated with samples collected by the various devices.

Results

The proportions of particles larger than 50 mm in diameter in samples other than those collected by the McNeil sampler were significantly related to the proportions in the test substrates. But the slopes of the regressions for the single-probe freeze-core samples ($b_1 = 2.75$) and triple-probe freeze-core samples ($b_1 = 2.28$) were both significantly different from unity. Therefore, we excluded particles larger than 50 mm in diameter from further analyses and recalculated the proportions of the remaining sizes for comparisons of proportions of individual particle sizes.

We found significant differences in the proportions of several particle sizes between test substrates and the samples collected by the four devices (Table 2). Single-probe freeze-core samples differed most often, while McNeil and shovel samples differed least often from the test substrates. All devices undersampled particles 6.3–9.5 mm and those less than 0.212 mm in diameter. Both freeze-core samplers oversampled particles 25–50 mm in diameter.

TABLE 2. Comparisons of proportions of substrate retained on sieves of different sizes between the test substrates and substrate samples collected by four devices. An inequality sign indicates a significant difference (Wilcoxon signed-rank test) and whether the proportion of that size class in the samples collected with each device tended to be greater or less than the proportion of that size class in the test substrates.

Sieve size (mm)	Device[a]			
	FC1	FC3	McN	Shv
25.0	>	>		
12.5				
9.5				
6.3	<	<	<	<
3.35	<		>	>
1.70	<	<		
0.85	<			
0.425				
0.212		>		
<0.212	<	<	<	<

[a]FC1 and FC3, single-probe and triple-probe freeze-core samples, respectively; McN = McNeil samples; Shv = shovel samples.

TABLE 3. Comparisons of proportions of substrate retained on sieves of different sizes among substrate samples collected by four devices. An asterisk indicates that the overall distributions of a particular substrate size among samples were significantly different (Kruskal–Wallis two-way analysis of variance); an inequality sign indicates a significant difference (nonparametric Tukey test) and whether the proportion of that size class in the samples collected with the upper device tended to be greater or lesser than the proportion of that size class in samples collected with the lower device.

Sieve size (mm)	Devices[a]					
	FC1: FC3	FC1: McN	FC1: Shv	FC3: McN	FC3: Shv	McN: Shv
25.0*						
12.5*						
9.5						
6.3*			<	<	<	
3.35*		<	<	<	<	
1.70*		<	<	<	<	
0.85*						
0.425						
0.212						
<0.212*			>		>	

[a]FC1 and FC3, single-probe and triple-probe freeze-core samples, respectively; McN = McNeil samples; Shv = shovel samples.

Similarly, the proportions of at least one particle size collected by most of the devices differed significantly from those collected by at least one other device (Table 3). Differences between samples collected by the two types of freeze-core samplers were not significant, nor were differences between samples collected by McNeil samplers and shovels. The proportions of particles less than 0.212 mm in diameter were greater in freeze-core samples than in shovel samples. McNeil and shovel samples contained greater proportions of the intermediate sizes than did the freeze-core samples.

Finally, the arithmetic means of geometric mean particle sizes of samples differed significantly from the geometric mean particle sizes of several of the test substrates (Table 4). Also, 28 of the 32 sample geometric mean particle sizes exceeded those of the test substrates. The samples collected by the single-

TABLE 4. Arithmetic means (and standard errors) of geometric mean particle sizes (mm) of samples collected by four devices from 10 substrates of varying composition. Substrate is the test substrate label and its geometric mean. An asterisk indicates a significant difference (t-test) between test and sample geometric mean particle sizes at $P \leq 0.05$.

Test substrate	Device[a]			
	FC1	FC3	McN	Shv
A 3.6	14.1	6.8	—	—
	(8.1)	(2.8)		
B 3.9	—	—	5.9	7.0
			(2.3)	(2.1)
C 9.9	23.7	20.9*	—	—
	(10.2)	(4.3)		
D 9.9	—	—	8.9	14.7*
			(1.5)	(1.3)
E 1.5	2.7	2.6*	2.3*	2.3*
	(0.9)	(0.4)	(0.3)	(0.5)
F 4.7	15.3*	7.8	4.3	6.5
	(6.2)	(2.3)	(1.6)	(2.0)
G 3.3	6.2	4.6*	3.1	4.0
	(5.0)	(0.5)	(0.6)	(0.5)
H 4.8	4.7	5.0	5.0	5.3
	(0.8)	(0.7)	(0.2)	(0.5)
I 11.8	17.4	17.2	13.6	15.2
	(8.2)	(4.7)	(3.4)	(4.5)
J 6.7	13.3*	11.6*	8.9	7.1
	(3.9)	(1.9)	(1.9)	(1.8)

[a]FC1 and FC3; single-probe and triple-probe freeze-core samples, respectively; McN = McNeil samples; Shv = shovel samples.

probe freeze-core sampler generally had the largest and most variable geometric mean particle sizes. In contrast, geometric mean particle sizes of McNeil samples usually were comparable with those of the test substrates. Regressions between geometric mean particle sizes of samples and test substrates corroborated these findings. The coefficients of determination of the regressions between the geometric mean particle sizes of the test substrates and those of the samples collected by the single-probe freeze core device, the triple-probe freeze-core device, the McNeil sampler, and the shovel were 0.36, 0.76, 0.73, and 0.81, respectively.

Discussion

The composition of substrate samples collected by all devices often differed from the composition of test substrates, both in the proportions of individual particle sizes and in geometric mean particle sizes. These differences indicate inaccuracies associated with the samplers, especially with respect to the larger particles, but may also suggest problems with the sampling techniques. For example, the insertion of all samplers disturbed the substrate. Such disturbance could cause fine sediment to be displaced deeper into the stream bottom or downstream by the current and could lead to undersampling of these small particles. Field studies (Everest et al. 1982; Young et al. 1989) reported that fine sediment increased with depth in the substrate, but this could be partly attributable to infiltration associated with sampling. A related problem involves the loss of fine sediment as a sample is removed from the stream bottom. Because we sampled from standing water, we could not estimate the amount of sediment that would be detached from samples by running water. However, one could alleviate this problem by installing a portable stilling well (such as a bucket without a bottom) to surround a portion of the stream bottom and effectively eliminate flow prior to sampling (T. C. Bjornn, University of Idaho, Moscow, ID, pers. comm.).

Both freeze-core samplers, particularly the single-probe sampler, tended to oversample large particles. Only in the test substrate lacking particles larger than 25 mm in diameter (test substrate H) did the average geometric mean particle size of the single-probe samples underestimate the geometric mean particle size of the test substrate. Adams and Beschta (1980) also reported that the single-probe freeze-core sampler was biased in favor of large particles; they hypothesized that only a small portion of a large particle needed to be frozen to the probe to be sampled. Lotspeich and Reid (1980) suggested that the triple-probe freeze-core sampler would overcome this problem, but we found that it also oversampled large particles.

We found little difference in the composition of substrate samples collected with single- or triple-probe freeze-core samplers or of samples collected with the McNeil sampler or shovel. Again, this observation conflicts with the suggestion that the triple-probe freeze-core sampler should collect less biased samples than the single-probe device (Everest et al. 1980). Differences in the samples collected by dissimilar samplers also contrast with the suggestion that freeze-core and McNeil samples were similar (Everest et al. 1980; Lotspeich and Reid 1980; Shirazi et al. 1981). However, Ringler (1970) also reported differences between freeze-core and McNeil samples.

The variability in composition of substrate samples taken by the triple-probe freeze-core sampler, McNeil sampler, and shovel was moderate, but the high variability of single-probe freeze-core samples reduces the value of this technique for field surveys (cf. Shirazi et al. 1981). For example, Adams and Beschta (1980) reported large spatial and temporal variation in the composition of stream substrates, but they did not quantify the proportion of that variation caused by their use of a single-probe freeze-core sampler. Crisp and Carling (1989), also using this device, failed to detect significant differences in substrate composition at sites before and after spawning by brown trout (*Salmo trutta*), yet such differences have been caused by spawning brook trout (Young et al. 1989) and brown trout (Grost 1989).

Nonetheless, freeze-core samplers, especially multiple-probe instruments (v. Platts and Penton 1980), remain useful because only these devices allow the stratification of a sample and an assessment of changes in substrate composition with depth (Everest et al. 1980). Perhaps further refinements of these devices, such as a larger spread between probes, additional probes, or longer freezing intervals, could reduce their imprecision and bias.

On the basis of the relatively low variances associated with McNeil samples, and on the frequent proximity of their geometric mean particle sizes to those of the test substrates, we consider the McNeil sampler to be the most accurate device for assessing overall substrate composition. Furthermore, this accuracy might be improved by including a suction mechanism inside the sampling tube to improve the retention of fine sediment (Koski 1966) and by enlarging the tube diameter. Shovel samples were quite similar to McNeil samples and were the only samples that accurately estimated the proportion of large particles. Because sampling with a shovel is faster and easier, we recommend further laboratory and field comparisons of these two sampling techniques.

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References

ADAMS, J. N., AND R. L. BESCHTA. 1980. Gravel bed composition in Oregon coastal streams. Can. J. Fish. Aquat. Sci. 37: 1514–1521.

CARLING, P. A., AND N. A. READER. 1981. A freeze-sampling technique suitable for coarse river bed-material. Sediment. Geol. 29: 233–239.

CHAPMAN, D. W., D. E. WEITKAMP, T. L. WELSH, M. B. DELL, AND T. H. SCHADT. 1986. Effects of river flow on the distribution of chinook salmon redds. Trans. Am. Fish. Soc. 115: 537–547.

CRISP, D. T., AND P. A. CARLING. 1989. Observations on siting, dimensions and structure of salmonid redds. J. Fish Biol. 34: 119–134.

EVEREST, F. H., F. B. LOTSPEICH, AND W. R. MEEHAN. 1982. New perspectives on sampling, analysis, and interpretation of spawning gravel quality, p. 325–333. *In* N. B. Armantrout [ed.] Acquisition and utilization of aquatic habitat inventory information. Western Division, American Fisheries Society, Bethesda, MD. 376 p.

EVEREST, F. H., C. E. MCLEMORE, AND J. F. WARD. 1980. An improved tri-tube cryogenic gravel sampler. U.S. For. Serv. Pac. Northwest For. Range Exp. Stn., Portland, OR, Res. Note PNW-350: 8 p.

GROST, R. T. 1989. A description of brown trout redds in Douglas Creek, Wyoming. M.S. thesis, University of Wyoming, Laramie, WY. 156 p.

KOSKI, K V. 1966. The survival of coho salmon (*Oncorhynchus kisutch*) from egg deposition to emergence in three Oregon coastal streams. M.S. thesis, Oregon State University, Corvallis, OR. 84 p.

LOTSPEICH, F. B., AND F. H. EVEREST. 1981. A new method for reporting and interpreting textural composition of spawning gravel. U.S. For. Serv. Pac. Northwest For. Range Exp. Stn., Portland, OR, Res. Note PNW-369: 11 p.

LOTSPEICH, F. B., AND B. H. REID. 1980. Tri-tube freeze-core procedure for sampling stream gravels. Prog. Fish-Cult. 42: 96–99.

MCNEIL, W. J., AND W. H. AHNELL. 1960. Measurement of gravel composition of salmon stream beds. Fish. Res. Inst. University of Washington, Seattle, WA, Circ. 120: 7 p.

NETER, J., W. WASSERMAN, AND M. H. KUTNER. 1985. Applied linear statistical models, second edition. Irwin, Homewood, IL. 547 p.

PIMENTEL, R. A., AND J. D. SMITH. 1990. Biostat I: a univariate statistical toolbox. Version 2.0. Sigma Soft, Placentia, CA. 392 p.

PLATTS, W. S., AND V. E. PENTON. 1980. A new freezing technique for sampling salmonid redds. U.S. For. Serv. Intermountain For. Range Exp. Stn., Ogden, UT, Res. Pap. INT-248: 22 p.

RINGLER, N. H. 1970. Effects of logging on the spawning bed environment in two Oregon coastal streams. M.S. thesis, Oregon State University, Corvallis, OR. 96 p.

RINGLER, N. H., AND J. D. HALL. 1988. Vertical distribution of sediment and organic debris in coho salmon (*Oncorhynchus kisutch*) redds in three small Oregon streams. Can. J. Fish. Aquat. Sci. 45: 742–747.

SCRIVENER, J. C., AND M. J. BROWNLEE. 1989. Effects of forest harvesting on spawning gravel and incubation survival of chum (*Oncorchynchus keta*) and coho salmon (*O. kisutch*) in Carnation Creek, British Columbia. Can. J. Fish. Aquat. Sci. 46: 681–696.

SHIRAZI, M. A., AND W. K. SEIM. 1979. A stream systems evaluation: an emphasis on spawning habitat for salmonids. U.S. Environ. Protect. Agency, Corvallis, OR, EPA-600/3-79-109: 36 p.

SHIRAZI, M. A., W. K. SEIM, AND D. H. LEWIS. 1981. Characterization of spawning gravel and stream system evaluation, p. 277–278. *In* Proceedings of the conference on salmon spawning gravel: a renewable resource in the Pacific Northwest? Rep. 39, Washington Water Research Center, Washington State University, Pullman, WA.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. Freeman and Company, San Francisco, CA. 859 p.

STOWELL, R., A., ESPINOSA, T. C. BJORNN, W. S. PLATTS, D. C. BURNS, AND J. S. IRVING. 1983. Guide for predicting salmonid response to sediment yields in Idaho batholith watersheds. U.S. For. Serv. Northern and Intermountain Regions, Ogden, UT. 95 p.

WALKOTTEN, W. J. 1976. An improved technique for sampling streambed sediments. U.S. For. Serv. Pac. Northwest For. Range Exp. Stn., Portland, OR, Res. Note PNW-281: 11 p.

YOUNG, M. K. 1989. Effect of substrate composition on the survival to emergence of Colorado River cutthroat trout and brown trout. Ph.D. dissertation, University of Wyoming, Laramie, WY. 121 p.

YOUNG, M. K., W. A. HUBERT, AND T. A. WESCHE. 1989. Substrate modification by spawning brook trout in a southeastern Wyoming stream. Trans. Am. Fish. Soc. 118: 379–385.

Changes in Catchability in a Bottom-Trawl Fishery for Cape Hake (*Merluccius capensis*)

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Gordoa, A., and J. E. Hightower. 1991. Changes in catchability in a bottom-trawl fishery for Cape hake (*Merluccius capensis*). Can. J. Fish. Aquat. Sci. 48: 1887–1895.

Management of the Cape hake (*Merluccius capensis*) stock off the coasts of Angola and Namibia has been based on surplus production modeling, which incorporates an assumption of constant catchability. Results of least-squares catch-at-age analysis and virtual population analysis were used to estimate catchability from 1968 to 1986. Estimated catchability was either relatively constant or gradually increasing from 1968 to 1978, but fluctuated considerably between about 1979 and 1983. The fluctuations may have been due to an inverse relationship between catchability and abundance or to environmental factors affecting fish distribution. The period of highest estimated catchability corresponded to the period of lowest estimated abundance and below-average water temperatures. If management is based on the assumption of constant catchability but catchability is inversely related to abundance, the risk of overfishing will be greatest when the stock is at the lowest levels. Future assessments should place increased reliance on survey data or incorporate an appropriate model of catchability changes.

La gestion du stock de merlu du Cap (*Merluccius capensis*) de la côte de l'Angola et de la Namibie s'est fondée sur la modélisation de la production excédentaire, avec un potentiel de capture supposé constant. Pour estimer le potentiel de capture, de 1968 à 1986, on s'est servi de l'analyse des prises par classes d'âge par la méthode des moindres carrés et de l'analyse de la population virtuelle. Le potentiel de capture estimatif a été, soit relativement constant, soit graduellement croissant, de 1968 à 1978, mais il a considérablement fluctué entre 1979 et 1983. Les fluctuations observées étaient peut-être dues à une relation inverse entre le potentiel de capture et l'abondance ou à l'influence de facteurs du milieu sur la distribution des poissons. La période où le potentiel de capture estimé était le plus élevé correspond à la période où la valeur estimative de l'abondance était la plus faible et les températures de l'eau, sous la moyenne. Lorsque la gestion se base sur un potentiel de capture supposé constant et que ce potentiel est une fonction inverse de l'abondance, le risque de surpêche est maximal lorsque le stock est au plus bas. À l'avenir dans les évaluations, il faudrait se fier davantage aux résultats des relevés ou utiliser le modèle approprié, avec variations de potentiel de capture.

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A substantial fishery for Cape hake (*Merluccius capensis*) has existed off the coasts of Angola and Namibia since 1965. Landings from latitude 15 to 25°S (statistical divisions 1.3 and 1.4 of the International Commission for the Southeast Atlantic Fisheries (ICSEAF)) ranged from 90 500 tonnes (t) in 1980 to 606 100 t in 1972 (Table 1). Catches are made primarily with bottom-trawl gear although some catches are made at night in a midwater-trawl fishery (Crawford et al. 1987) for species such as horse mackerel (*Trachurus* spp.).

Both a surplus production model (Graham 1935) and virtual population analysis (VPA) (Murphy 1965) have been used to assess the status of the hake stock. Effort data used in the surplus production models were obtained from average weighted catch-per-unit-effort (CPUE) data, specifically tonnes per hour fished by Spanish bottom trawlers in tonnage class 7 (1000–1999 GRT). This weighted CPUE index, based on total catch and effort by fleet, is equivalent to using power factors to adjust for differences in catch rate (Andrew 1986).

A potential limitation of the surplus production model used to manage the hake fishery is the assumption that catchability (q) is constant. That assumption is used to relate observed fishing effort in year y ($f(y)$) to fishing mortality ($F(y)$):

$$F(y) = qf(y).$$

Based on that assumption, CPUE and abundance should be linearly related and management could be based on the CPUE data alone. The assumption that catchability is constant has been shown to be invalid for a number of pelagic species (Abramson and Tomlinson 1972; Fox 1974; MacCall 1976; Peterman and Steer 1981; Shelton and Armstrong 1983; Winters and Wheeler 1985) because of the interaction between fishermen and schooling fishes. Search time is an important component of total effort in many pelagic fisheries whereas the catch per unit of fishing time may simply reflect the within-school density (Paloheimo and Dickie 1964). If within-school density is maintained as abundance decreases, there would be an inverse relationship

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TABLE 1. Catch (thousands of tonnes) and standardized fishing effort (estimated hours fished in ICSEAF divisions 1.3 and 1.4 by Spanish bottom trawlers in tonnage class 7 (1000–1999 GRT)) (Draganik and Sacks 1987).

Year	Catch	Effort
1965	93.5	52 500
1966	212.4	162 500
1967	195.0	214 000
1968	382.7	399 000
1969	320.5	364 000
1970	402.5	447 000
1971	365.6	420 000
1972	606.1	842 000
1973	377.6	662 456
1974	318.8	708 400
1975	309.4	737 000
1976	369.8	880 476
1977	277.5	566 300
1978	258.1	600 200
1979	172.3	430 700
1980	90.5	201 100
1981	92.1	167 500
1982	176.4	332 800
1983	215.8	372 100
1984	228.5	357 000
1985	211.5	320 450
1986	230.9	355 230

between abundance and catchability. An inverse relationship also has been detected for several demersal species (Pope and Garrod 1975; Houghton and Flatman 1980; Angelsen and Olsen 1987; Crecco and Overholtz 1990), and it has been suggested that depensatory fishing mortality at low abundance levels is a general phenomenon among most commercial fisheries (Winters and Wheeler 1985; Crecco and Overholtz 1990).

Radovich (1982) noted that catchability will be constant only if (1) fishes are uniformly distributed over the fishing area, (2) effort is distributed randomly over the fishing area, and (3) units of effort are independent. The first assumption is unlikely to be true for Cape hake because schooling behavior has been observed for fish younger than age 2, and older fish form aggregations and migrate to spawning grounds (Shannon and Pillar 1986). The latter two assumptions are almost certainly violated because fishermen work cooperatively to locate and exploit areas of good catches. A preliminary analysis of catch-at-age data from ICSEAF division 1.5 provides evidence that catchability may be an inverse function of biomass (Gordoa and Pertierra 1987). The reliability of those results is unknown, however, because of a limitation in the catch data. Division 1.5 catches contain two hake species (*M. paradoxus* and *M. capensis*) that are quite similar in appearance; unfortunately, the proportion of the two species in historical commercial catches is not known.

The objective of this study was to examine the constant catchability assumption for the Cape hake fishery in divisions 1.3 and 1.4. We used results from VPA and least-squares analysis (Deriso et al. 1985) of the catch-at-age data to estimate fishing mortality rates and catchabilities. Observed trends in catchability were compared with estimates of abundance and information on environmental changes in order to develop hypotheses about the causes of changes in catchability.

Methods

Catch-at-age data for ages 1–10 were available for 1968–86 (Table 2). Catches of fish older than age 10 were negligible. The age composition estimates were obtained through an at-sea sampling program in which three to four observers measured about 100 000 fish and collected about 5000–6000 otoliths annually. The smallest hake require more time to process and are less valuable; consequently, catches of age 1 hake were underestimated prior to 1973 due to discarding. Discarding is believed to have been negligible in more recent years because of the relatively lower catches of older fishes.

Estimates of fishing mortality rates and catchability coefficients were based on results of VPA and least-squares catch-at-age analysis. The primary advantage of the VPA, which was done by Draganik and Sacks (1987), was that selectivity patterns could vary from year to year. The selectivity pattern for the least-squares analysis was assumed to be constant over selected ranges of years. Because of that simplifying assumption, an advantage of the least-squares analysis was that many fewer parameters had to be estimated. Another advantage was the assumption that catch and effort were measured with error; thus, residuals could be examined for evidence of lack of fit due to violations in the model assumptions (e.g. trends in the effort residuals due to changes in catchability).

The methods used by Draganik and Sacks (1987) in the VPA can be summarized as follows. A linear regression of the average F for ages 1–7 versus standardized fishing effort (from the weighted average CPUE index described above) was fitted using VPA results for 1968–82. The resulting regression equation was used to predict average F's for 1982–86. The selected starting F for the VPA was the value that minimized the squared difference between the 1982–86 average F's for ages 1–7 and the values predicted from the regression equation. (The starting F for age 10 fish was assumed to be constant over all years. The starting F's in 1986 for ages less than the assumed age at full recruitment (age 4) were based on an assumed selectivity pattern.) A natural mortality rate (M) of 0.3 was assumed for all ages and years.

The least-squares model that we used (Deriso et al. 1985) was based on the following assumptions: (1) fishing mortality could be separated into an age-dependent factor (selectivity) and a year-dependent factor (the fishing mortality rate for fully recruited fish, $F(y)$), (2) catchability was constant and predicted fishing effort ($f'(y)$) was obtained as $F(y)/q$, and (3) errors in measuring catch and fishing effort were lognormally distributed. The microcomputer program CAGEAN (Deriso et al. 1985) was used to estimate the model parameters. As in the VPA, we assumed that M was 0.3 for all ages and years (Draganik and Sacks 1987).

Several model parameters were varied to evaluate the model's sensitivity to assumptions about catchability and selectivity. We varied the weighting factor (λ) used to adjust the amount of influence given the auxiliary information on fishing effort (Table 1). A low λ value (1.0) permitted predicted and observed effort to deviate considerably whereas a larger value (10.0) forced relatively close agreement (and adherence to the constant catchability assumption). We were unable to obtain solutions for λ values greater than 10, suggesting an inconsistency between the catch-at-age and effort data.

We evaluated three alternative assumptions about the range of years for which a constant selectivity pattern was assumed to apply: (1) a single selectivity period from 1968 to 1986, (2) two selectivity periods during which trawl mesh size was 70–120 mm (1968–75), or a minimum of 110 mm (1976–86) (Andrew and Butterworth 1988) and (3) selectivity periods 1968–81 and 1982–86 corresponding to the periods before and

TABLE 2. Number of Cape hake caught (in millions) in ICSEAF divisions 1.3 and 1.4 (from Draganik and Sacks 1987).

	Age									
Year	1	2	3	4	5	6	7	8	9	10
1968	2.72	89.83	561.14	193.77	74.17	11.64	2.45	0.00	0.00	0.00
1969	0.00	52.98	231.41	212.35	59.27	17.16	8.33	2.50	1.33	0.00
1970	0.13	128.47	320.82	203.83	110.20	21.97	7.37	1.15	0.67	0.00
1971	0.03	24.84	161.14	264.95	72.17	21.73	11.41	3.64	2.08	0.50
1972	2.91	48.67	549.59	346.37	119.41	38.32	19.59	3.68	1.36	0.63
1973	27.78	53.23	383.21	290.85	48.42	10.52	5.42	6.60	1.25	0.91
1974	32.41	162.77	160.17	60.34	103.79	57.22	15.27	2.62	1.28	0.69
1975	26.78	126.94	362.41	114.99	32.96	18.88	13.88	6.74	3.61	1.89
1976	71.41	345.29	500.98	120.59	14.23	12.90	2.25	1.69	0.80	0.40
1977	51.58	94.33	225.85	181.45	28.39	9.80	7.14	4.65	1.24	1.35
1978	48.49	434.71	307.54	74.92	36.89	11.07	1.69	0.38	0.64	0.11
1979	18.77	113.85	121.15	66.95	23.25	12.85	5.47	3.97	1.62	1.75
1980	44.05	36.19	49.52	40.41	12.89	9.86	4.08	1.35	0.64	0.76
1981	25.15	45.83	45.38	34.39	25.90	11.23	2.24	1.67	0.26	0.06
1982	135.80	312.31	168.97	47.96	13.30	7.34	4.75	2.65	1.01	0.46
1983	872.13	739.33	97.18	42.68	11.61	5.21	2.10	0.80	0.32	0.20
1984	34.76	667.31	295.07	54.26	17.00	5.88	1.63	1.42	0.11	0.76
1985	56.49	127.01	230.17	129.24	31.79	5.45	1.25	0.74	0.15	0.01
1986	25.50	301.40	135.40	114.50	69.10	20.10	10.40	2.50	0.80	0.00

after the entry of the apparently strong 1981 and 1982 year classes (i.e. targeting on strong year classes). We also obtained some estimates under the assumption that a range of ages (e.g. 3–5 or 4–6) were equally vulnerable to fishing; the results were similar to those reported here.

We used results from VPA to determine whether there had been obvious changes in selectivity patterns over time. Because the starting F's would have greatest impact on estimates for the oldest age groups, we calculated VPA selectivity estimates (i.e. age-specific F's for each year scaled to a maximum of 1.0) using F's for ages 1–7 only.

By varying the assumptions used in the least-squares analysis, we obtained a wide range of estimated population levels and fishing mortality rates. These solutions appeared to be about equally consistent with the observed catch data; consequently, we rejected some solutions by using the following two alternative sources of recruitment estimates.

(1) Annual trawl survey estimates of absolute abundance have been obtained each August since 1983 using the swept-area method, based on 20 d of trawling with four to five tows per day at randomly selected sites (Macpherson et al. 1985). These surveys were conducted over latitudes 23–30°S, compared with 15–25°S for the commercial catch data. Because of the lack of overlap and difference in coastline length, we used the survey estimates only to rule out extreme solutions.

(2) Based on the convergence property of VPA, we felt that the VPA estimates of recruitment for the 1968–77 year classes should be relatively reliable. For those year classes, the estimated abundance at age 1 was based on a starting F at age 10. The cumulative F for ages 1–9 exceeded 3.5 for each year class, which should be more than sufficient to insure convergence. The VPA estimate of mean recruitment from 1968 to 1977 was 1.67 billion recruits using a starting F of either 0.65 or 0.75 (Draganik and Sacks 1987). Based on those results, we believe that a mean 1968–77 recruitment level substantially different from 1.67 billion is unlikely at the assumed level of natural mortality.

For the catch-at-age analyses, we estimated the approximate overall catchability in year y ($q(y)$) as

$$q(y) = C(y)/(f(y)B_{exp}(y))$$

where $C(y)$ was the catch biomass and $B_{exp}(y)$ was the estimated exploitable biomass in year y. For VPA, we used the estimated selectivity patterns for ages 1–7 (described above) to determine exploitable biomass. Thus, our VPA estimate of catchability applied to ages 1–7 only. Crecco and Overholtz (1990) estimated catchability using total biomass; however, we used exploitable biomass in an attempt to separate selectivity and catchability changes.

Although constant catchability is assumed in the CAGEAN program, estimated catchability will vary among years due to differences between observed and predicted effort unless λ is large. Crecco and Overholtz (1990) used survey estimates of abundance and VPA estimates of catchability to test for a nonlinear relationship between abundance and catchability. In our study, both abundance and catchability were estimated using the catch-at-age data; consequently, no significance tests were carried out.

We examined the 23–30°S survey data for evidence of recent (1983–89) changes in the area occupied by *M. capensis*. For each year, we plotted cumulative distributions showing the proportion of total biomass from each survey stratum. A contraction in the area occupied by the stock would cause a larger proportion of the stock to occur in fewer strata.

Results

Estimated Abundance and Selectivity

The least-squares recruitment estimates were most consistent with the survey and VPA estimates and least sensitive to λ for the case where selectivity was assumed to have changed during 1982–86 (relative to 1968–81) in order to target on the apparently strong 1981 and 1982 year classes (Table 3). We estimated that fish ages 1–2 had increased vulnerability to

TABLE 3. Recruitment (billions of age 1 fish) as estimated by VPA (Draganik and Sacks 1987), the swept-area method using trawl survey catches (Macpherson and Roel 1984), and three least-squares analyses using different assumptions about selectivity. λ represents the relative weight given the auxiliary information on fishing effort in the least-squares analyses.

			Selectivity period					
			1968–86		1968–75, 1976–86		1968–81, 1982–86	
Year	VPA	Survey	λ = 1	λ = 10	λ = 1	λ = 10	λ = 1	λ = 10
1968	2.23		2.77	49.43	2.60	25.54	2.85	1.54
1969	2.10		1.21	21.02	1.19	10.54	1.25	0.79
1970	2.61		1.59	26.65	1.85	13.99	1.65	1.24
1971	1.33		0.93	15.68	1.15	8.36	0.94	0.78
1972	0.90		1.08	17.91	1.45	10.23	1.12	0.97
1973	1.60		2.21	36.05	2.86	21.74	2.30	2.11
1974	2.04		2.71	45.05	2.69	26.94	2.73	2.39
1975	1.45		2.53	42.41	2.49	22.39	2.49	2.01
1976	1.20		1.52	25.87	1.06	8.81	1.57	1.18
1977	1.26		1.63	29.21	0.99	9.40	1.77	1.09
1978	0.55		1.46	32.40	0.89	9.75	1.11	0.72
1979	0.44		1.15	29.58	0.65	8.14	0.63	0.52
1980	0.84		2.27	56.00	1.21	14.83	1.18	1.15
1981	1.20		3.37	80.79	1.73	19.45	2.10	1.84
1982	2.82		9.15	213.11	4.24	45.75	3.82	2.64
1983	3.18	3.75	17.74	376.05	6.69	64.70	5.86	3.49
1984	0.84	5.53	5.90	111.52	1.65	13.58	1.23	0.69
1985	1.42	5.38	21.29	343.04	3.48	25.79	2.25	1.21
1986	0.51	4.68	12.82	202.11	0.97	7.34	0.66	0.33
1987		2.51						
1988		1.46						
1989		2.21						

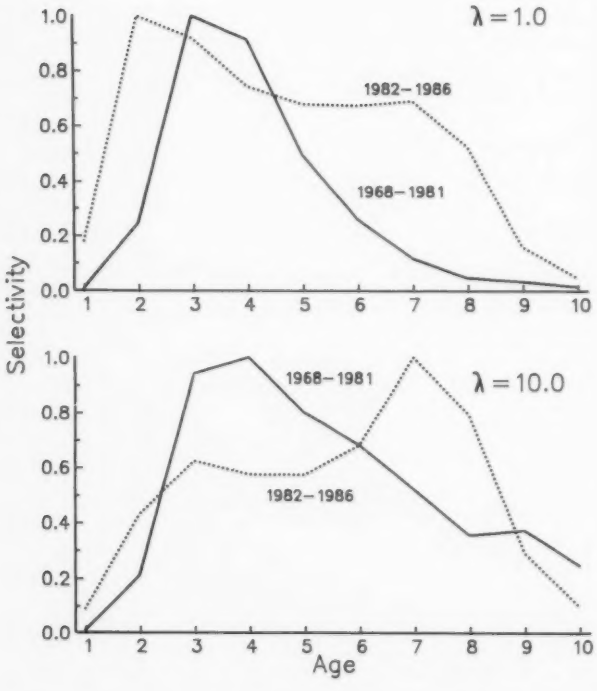

FIG. 1. Least-squares estimates of the selectivity patterns for periods 1968–81 and 1982–86. λ represents the relative weight given the auxiliary information on fishing effort.

fishing during 1982–86 relative to 1968–81 (Fig. 1); thus, the large recent catches were attributed both to strong recruitment and selectivity changes. The increased vulnerability of fish ages 1–2 also was evident in the 1982–85 selectivity patterns based on the VPA results (Fig. 2). There were no apparent trends in the least-squares catch residuals for this case, except that the predicted catches of age 1 fish were much larger than the observed catches during 1969–71 (i.e. years in which discarding was thought to occur.

When a single selectivity period (1968–86) was assumed, the large catches during 1982–86 were attributed solely to strong incoming year classes. The estimates of 1982–86 recruitment were considerably higher than either the survey or VPA estimates (Table 3). When selectivity periods 1968–75 and 1976–86 were assumed (before and after the minimum mesh regulation was implemented), recruitment estimates for λ = 1.0 were similar in magnitude to the survey and VPA estimates whereas the results for λ = 10.0 were substantially higher (Table 3). As in the previous case, recruitment estimates generally were highest during 1982–86. We did not find evidence in our VPA selectivity estimates (Fig. 2) of a consistent change in selectivity due to the minimum mesh regulation. Based on these results, we limit subsequent discussion of the least-squares analysis to the case where selectivity periods 1968–81 and 1982–86 were assumed.

The 1968 number-at-age vectors and resultant biomass estimates differed considerably for the two λ values (Fig. 3), although the biomass estimates converged somewhat by 1982 due to the similar estimates of recruitment during the 1970's (Fig. 4). The larger change in biomass from 1968 to 1982 corresponded to the larger change in predicted fishing effort, which was associated with the smaller λ value (Fig. 5). The

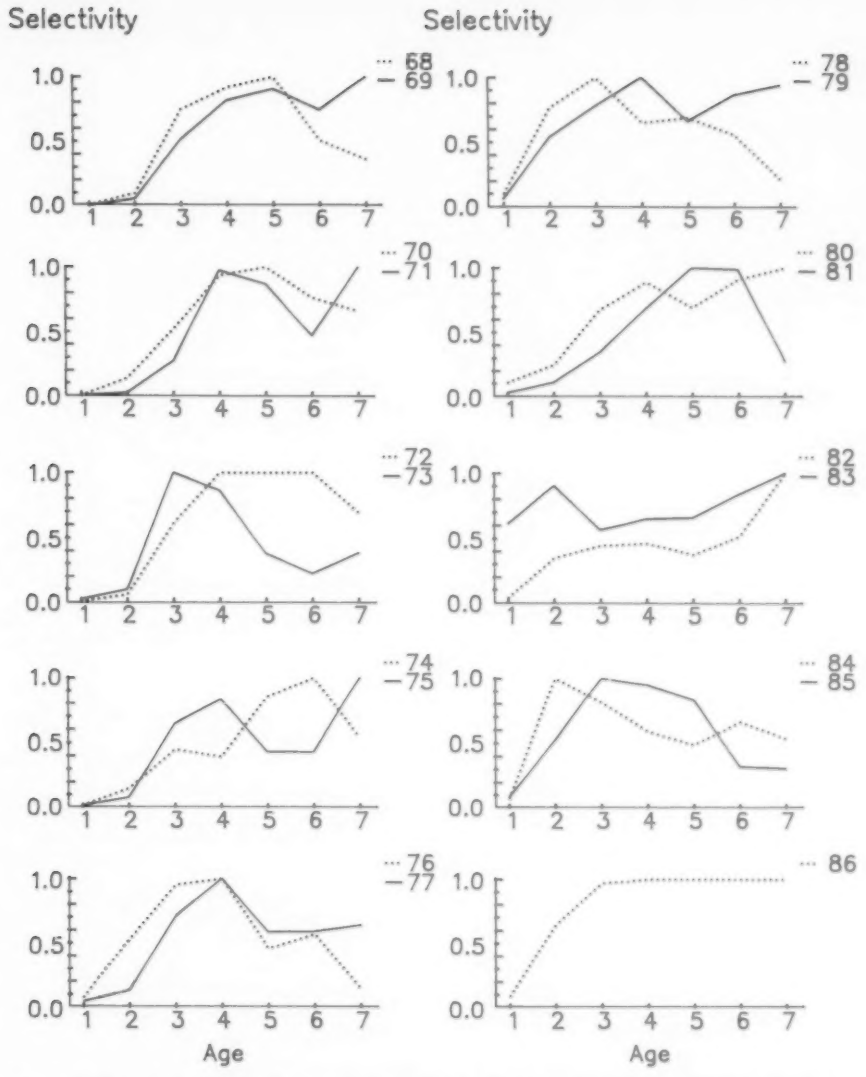

FIG. 2. Estimated annual selectivity patterns for fish ages 1–7, based on VPA results obtained by Draganik and Sacks (1987). Fishing mortality rates (F) for age 10 fish were assumed values. For that reason, F's for fish ages 8–10 were not used to calculate selectivities to reduce their impact on the estimated selectivity patterns.

change in biomass was much smaller when predicted and observed effort were forced to agree more closely.

Estimated Catchability Changes

The relative weight given the auxiliary information on fishing effort strongly affected the estimated F's, or equivalently, the predicted fishing effort levels (Fig. 5). When λ was low, predicted effort increased at a much faster rate from 1968 to 1979 than did observed effort. Observed and predicted effort levels were generally similar from 1983 to 1986.

Estimated catchability was relatively constant prior to about 1979 but fluctuated considerably between about 1979 and 1983 (Fig. 6). The decrease in catchability after 1981 corresponded to a period of increasing numerical abundance due to strong recent recruitment (Fig. 3). These changes resulted in an apparent inverse relationship between estimated abundance and catchability, particularly for the least-squares analysis with λ = 1.0 (Fig. 7). This relationship is highly tentative, however, because estimated total biomass appears as a variable on both axes of the catchability plot.

Changes in Spatial Distribution

The differences we observed in area occupied by *M. capensis* from 1983 to 1989 (Fig. 8) were not consistent with the changes in estimated biomass (Fig. 2). The apparent contraction in 1986 and 1987 did not persist in 1988 and 1989, despite further decreases in estimated biomass.

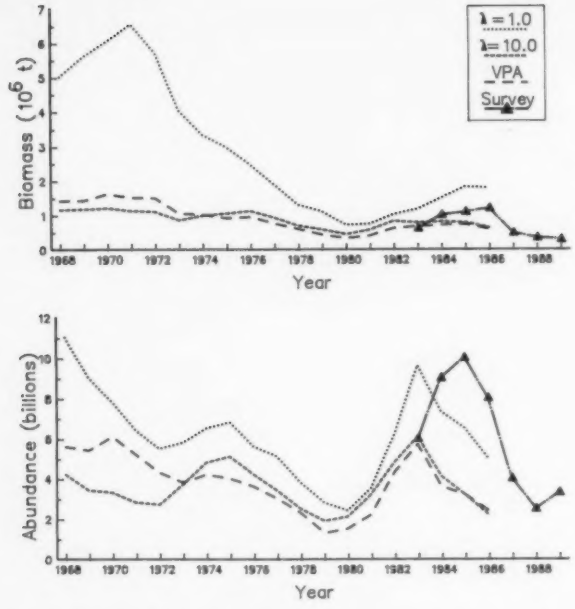

FIG. 3. Estimated levels of biomass and numerical abundance from 1968 to 1986, based on a least-squares catch-at-age analysis. λ represents the relative weight given the auxiliary information on fishing effort.

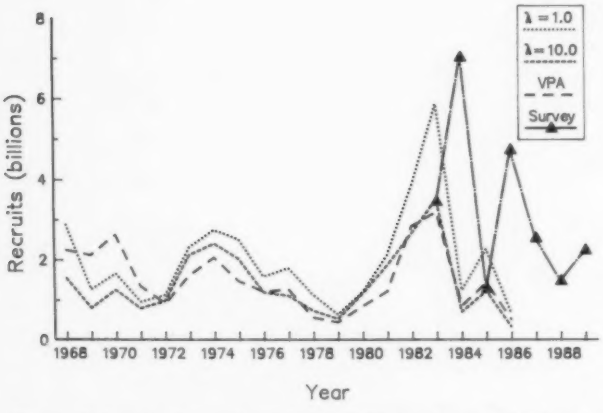

FIG. 4. Estimates of annual recruitment (billions of age 1 fish), based on a least-squares catch-at-age analysis (where λ represents the relative weight given the auxiliary information on fishing effort), VPA (Draganik and Sacks 1987), and swept-area estimates from 1983 to 1989 trawl surveys at latitudes 23–30°S (Macpherson and Roel 1984).

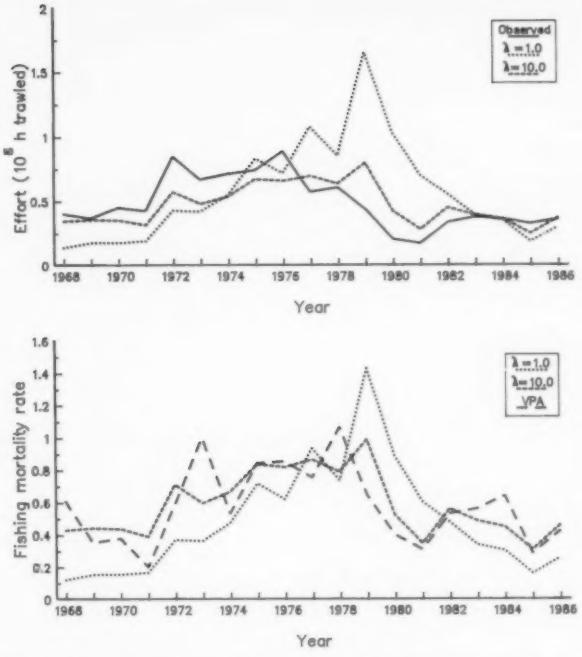

FIG. 5. Upper panel: observed fishing effort (Draganik and Sacks 1987) and predicted levels based on least-squares catch-at-age analysis. Lower panel: fishing mortality rates obtained from least-squares catch-at-age analysis and from VPA (Draganik and Sacks 1987). λ represents the relative weight given the auxiliary information on fishing effort in the catch-at-age analyses.

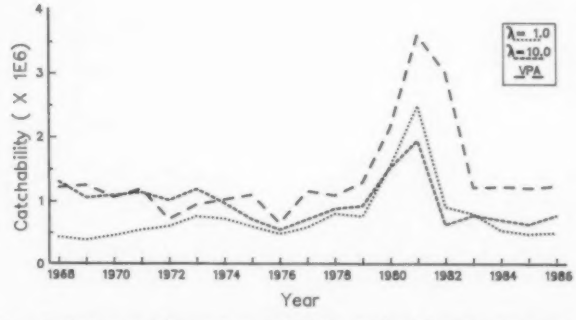

FIG. 6. Estimates of annual catchabilities obtained from least-squares catch-at-age analysis (where λ represents the relative weight given the auxiliary information on fishing effort) and from VPA (Draganik and Sacks 1987). Catchability estimates for VPA represent ages 1–7 only.

Discussion

Our recruitment estimates were highly sensitive to the assumption that the selectivity pattern was constant over time, and we obtained unreliable estimates for two of the three cases we examined. Our results supported earlier studies indicating that a model with more than one selectivity period was required (Butterworth et al. 1986a) and that the mesh regulation did not change overall catchability significantly (Andrew and Butterworth 1988). Butterworth et al. (1986a) examined the hypothesis that selectivity for young fish increased gradually from 1968 to 1983, due perhaps to changes in the spatial distribution of fishing effort. They concluded that the changes in catch-at-age data appeared more complex than could be accounted for by the selectivity submodel that they assumed. The results obtained in our study (based on three additional years of catch data) appear to support selectivity changes in response to fluctuations in year-class strength (i.e. targeting on strong year classes).

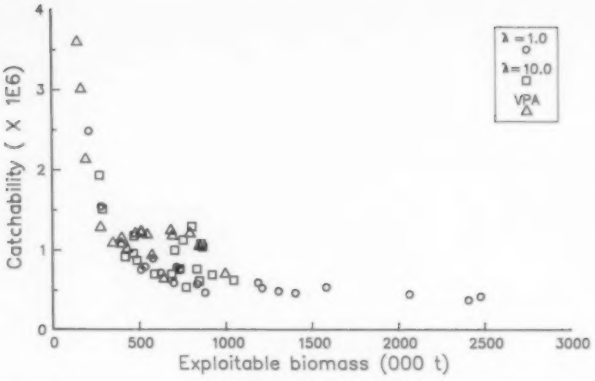

FIG. 7. Estimates of catchability versus exploitable biomass obtained from least-squares catch-at-age analysis (where λ represents the relative weight given the auxiliary information on fishing effort) and from VPA (Draganik and Sacks 1987). Catchability and exploitable biomass estimates for VPA represent ages 1–7 only.

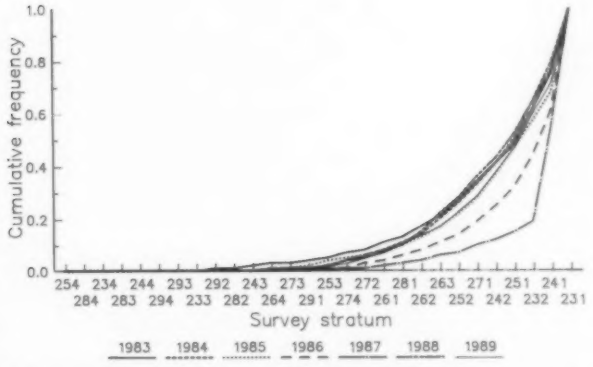

FIG. 8. Cumulative proportion of total biomass accounted for annually by each sampling stratum, based on trawl survey estimates of abundance for the area 23–30°S. Sampling strata are ordered from lowest to highest 1983–89 average catch rate.

The selectivity patterns from the least-squares analysis were sensitive to λ for 1982–86. Estimates for the latter selectivity period should stabilize over time as additional years of data are included in the analysis. For three of the four selectivity patterns, vulnerability to fishing decreased at older ages (Fig. 1). A similar trend was apparent in some of the VPA selectivity patterns, although those patterns varied substantially from year to year (Fig. 2). Decreased vulnerability for older fish may be reasonable because (1) vulnerability to trawl gear decreases at older ages for some species (e.g. Pacific whiting (*M. productus*), Hollowed et al. 1988; sablefish (*Anoplopoma fimbria*), Methot and Hightower 1988) and (2) Cape hake older than age 10 occur rarely in trawl catches but have been caught in exploratory fishing with hook-and-line gear.

An advantage of this and earlier catch-at-age analyses was the ability to identify alternative population trends that could account for the observed catch and effort patterns. In a traditional surplus production model, the recent increases in CPUE would be attributed to large increases in recruitment. In the catch-at-age analyses, the CPUE increases would be attributed either to strong recruitment or to moderate recruitment and changing selectivity and catchability. Because of discrepancies between results from surplus production and age-structured models, Butterworth et al. (1986a) hypothesized that the CPUE increases may have been due partly to a change in the spatial distribution of fishing effort. They suggested that the increased catches of young fish may have been due to increased fishing in inshore areas inhabited by younger hake. Our results suggest that the CPUE increases were due to a combination of changes in selectivity, catchability, and the strength of incoming year classes.

Estimated catchability generally increased from 1968 to 1981 in the catch-at-age analysis for the lower λ value (Fig. 6) because catchability changes could occur as a trend in the effort residuals. Those changes in estimated catchability would be consistent with increasing fishing power, due perhaps to increased knowledge of the fishing grounds or improvements in gear. For the least-squares analysis using the higher λ value, catchability was more constrained to fluctuate about a constant level.

If catchability increased gradually over time, then recent quotas derived from surplus production modeling would be overestimated (Butterworth and Andrew 1987). However, the fluctuations in catchability observed in all cases from about 1979 to 1983 suggested that factors other than changes in fishing power contributed to the observed trends. The temporal changes in estimated biomass and numerical abundance (Fig. 3) resulted in an apparent inverse relationship between estimated catchability and abundance (Fig. 7). Crecco and Overholtz (1990) noted a similar relationship for Georges Bank haddock (*Melanogrammus aeglefinus*), which they attributed to changes in the area occupied by the stock.

One explanation for an inverse relationship between catchability and abundance is that fish populations tend to contract toward the most favorable habitat as they are fished down (MacCall 1990). We did observe a substantial apparent contraction using the approach proposed by Crecco and Overholtz (1990) (for each year, summing the area of strata for which the catch rate exceeded the long-term median). However, based on the cumulative distribution plots (Fig. 8), we inferred that the decreases in stratum catch rates represented overall decreases in abundance. The apparent changes in spatial distribution for the area surveyed (23–30°S) (Fig. 8) were not consistent with changes in abundance in areas 1.3 and 1.4, but could be due to changes in other factors such as temperature or food availability. We are unable to determine whether the stock contracted during the period of highest estimated catchability because of the absence of survey data prior to 1983.

Changes in water temperature could have affected catchability. The period from 1979 to 1983 was one of below-average sea-surface temperatures (SST) for the Benguela system (McLain et al. 1985). Based on monthly mean SST for 3° blocks, anomalies of less than −0.5°C were observed during most of 1979–83, with greatest negative anomalies (less than −1°C) during November 1981 to May 1982 (McLain et al. 1985). Using SST data through 1984 in a modified catch-effort model, Butterworth (1988) reported a significant temperature effect on catchability assuming a 1-yr lag but none if a 0-yr lag was assumed.

If catchability is inversely related to abundance, management problems would be greatest when the stock declined to low levels: (1) biomass and catch quotas obtained from traditional surplus production models would tend to be overestimated

(because catchability would be higher than the historical mean) and (2) further management errors would be likely when setting either effort or catch quotas because of the rapidly changing relationship between effort and fishing mortality (Gulland 1977; Winters and Wheeler 1985).

We agree with Butterworth (1988) that conservative management is warranted until the factors affecting catchability can be reliably determined. Our results suggest that catchability and selectivity have changed over time; however, the apparent changes in catchability would also bias our estimates of it. Less biased estimates of catchability (and abundance) could presumably be obtained by using survey estimates of abundance to constrain the catch-at-age analysis (Winters and Wheeler 1985; Butterworth 1988). In our study, survey abundance estimates from an overlapping area were compared with the results of catch-at-age analysis and VPA. The survey estimates were useful for rejecting highly implausible solutions, but temporal changes in survey abundance were at odds with the VPA and catch-at-age analysis results (Fig. 3 and 4). A precise survey of areas 1.3 and 1.4, preferably measuring absolute abundance (Kimura 1989), would eliminate the need for potentially biased estimates of fishing effort. An additional advantage of survey data would be the opportunity to test for a relationship between catchability and abundance (Crecco and Overholtz 1990).

An alternative approach for dealing with catchability changes over time would be to use both survey estimates of abundance and commercial fishing effort data, but to vary the weighting factors to determine the sensitivity of population trends to the two sources of auxiliary data. Another alternative, for either a surplus production or catch-at-age model, would be to define catchability as a function of stock size or age structure or a constant that differed among periods (Fox 1974; Butterworth et al. 1986b; Deriso et al. 1989). Given the difficulty of exactly modeling catchability changes over time, that approach might be more effective if survey data were available to help constrain the catch-at-age analysis. Other approaches include analyzing catch and effort data on a finer spatial scale to separate changes in abundance from changed fishing distribution patterns and conducting a detailed reexamination of vessel power factors used to combine effort among fleets (Butterworth 1988).

In summary, our results suggest that catchability has varied over time, due perhaps to changes in fishing power, resource abundance, or oceanographic factors. Improved estimates of abundance and short-term yield can be obtained by incorporating precise survey data or an appropriate submodel of catchability changes into either a surplus production or age-structured model. Conservative management is warranted until factors affecting catchability can be identified.

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References

ABRAMSON, N. J., AND P. K. TOMLINSON. 1972. An application of yield models to a California shrimp population. U.S. Fish. Bull 70: 1021–1041.

ANDREW, P. A. 1986. Dynamic catch-effort models for the southern African hake populations. Ph.D. thesis, University of Cape Town. 248 p.

ANDREW, P. A., AND D. S. BUTTERWORTH. 1988. An examination of the effect of changes in mesh size on the catchability coefficient (q) and sustainable yield of the Southern African hake stocks. Collect. Sci. Pap. Int. Comm. SE Atl. Fish. 15(I): 23–45.

ANGELSEN, K. K., AND S. OLSEN. 1987. Impact of fish density and effort level on catching efficiency of fishing gear. Fish. Res. 5: 271–278.

BUTTERWORTH, D. S. 1988. Some recommendations regarding the assessment methodologies used by ICSEAF. Collect. Sci. Pap. Int. Comm. SE Atl. Fish. 15(I): 107–155.

BUTTERWORTH, D. S., AND P. A. ANDREW. 1987. Further results from the application of dynamic catch-effort models to the hake stocks in the ICSEAF convention area. Collect. Sci. Pap. Int. Comm. SE Atl. Fish. 14(I): 109–160.

BUTTERWORTH, D. S., M. O. BERGH, AND P. A. ANDREW. 1986a. A comparison of dynamic catch-effort model and VPA assessments for the hake stocks in ICSEAF divisions 1.3–1.6. Collect. Sci. Pap. Int. Comm. SE Atl. Fish. 13(1): 131–165.

BUTTERWORTH, D. S., M. O. BERGH, P. A. ANDREW, AND A. E. PUNT. 1986b. Some aspects of management strategies for and the assessment of the hake stocks off southern Africa. Collect. Sci. Pap. Int. Comm. SE Atl. Fish. 13(1): 167–193.

CRAWFORD, R. J. M., L. V. SHANNON, AND D. E. POLLOCK. 1987. The Benguela Ecosystem. Part IV. The major fish and invertebrate resources. Oceanogr. Mar. Biol. Annu. Rev. 25: 353–505.

CRECCO, V., AND W. J. OVERHOLTZ. 1990. Causes of density-dependent catchability for Georges Bank haddock *Melanogrammus aeglefinus*. Can. J. Fish. Aquat. Sci. 47: 385–394.

DERISO, R. B., P. R. NEAL, AND T. J. QUINN. 1989. Further aspects of catch-age analysis with auxiliary information, p. 127–135. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. Can. Spec. Publ. Fish. Aquat. Sci. 108.

DERISO, R. B., T. J. QUINN II, AND P. R. NEAL. 1985. Catch-age analysis with auxiliary information. Can. J. Fish. Aquat. Sci. 42: 815–824.

DRAGANIK, B., AND R. SACKS. 1987. Stock assessment of Cape hakes (*Merluccius capensis* and *M. paradoxus*) in the convention area. Divisions 1.3 + 1.4 Collect. Doc. Pap. Int. Commn SE. Atl. Fish. Doc. 18b.

FOX, W. W. 1974. An overview of production modelling, p. 142–156. *In* Proceedings of the ICCAT workshop on tuna population dynamics, Nantes, France, Sept. 1974.

GORDOA, A., AND J. P. PERTIERRA. 1987. Catch fluctuations observed in the Cape hake fishery in division 1.5. Collect. Sci. Pap. Int. Comm. SE Atl. Fish. 14(I): 207–218.

GRAHAM, M. 1935. Modern theory of exploiting a fishery and application to North Sea trawling. J. Cons. Int. Explor. Mer. 10: 264–274.

GULLAND, J. A. 1977. The stability of fish stocks. J. Cons. Int. Explor. Mer 37: 199–204.

HOLLOWED, A. B., R. METHOT, AND M. DORN. 1988. Status of the Pacific whiting resource in 1988 and recommendations to management in 1989. Appendix A in Status of the Pacific Coast groundfish fishery through 1988 and recommended acceptable biological catches for 1989. Pacific Fishery Management Council, Portland, OR.

HOUGHTON, R. G., AND S. FLATMAN. 1980. The exploitation pattern, density-dependent catchability, and growth of cod, *Gadus morhua*, in the west-central North Sea J. Cons. Int. Explor. Mer 39: 271–287.

KIMURA, D. K. 1989. Variability, tuning, and simulation for the Doubleday–Deriso catch-at-age model. Can. J. Fish. Aquat. Sci. 46: 941–949.

MACCALL, A. D. 1976. Density-dependence of catchability coefficient in the California Pacific sardine, *Sardinops sagax caerula*, purse-seine fishery. CalCOFI Rep. 18: 136–148.

1990. Dynamic geography of marine fish populations. University of Washington Press, Seattle, WA.

MACPHERSON, E., AND B. ROEL. 1984. Estudio del reclutamiento de la merluza en la divisions 1.4 y 1.5. Collect. Sci. Pap. Int. Comm. SE Atl. Fish. 11(1): 159–165.

MACPHERSON, E., B. ROEL, AND B. MORALES. 1985. Reclutamiento de la merluza y abundancia y distribución de diferentes especies comerciales en las divisiones 1.4 y 1.5 durante 1983–1984. Collect. Sci. Pap. Int. Comm. SE Atl. Fish. 12: 1–61.

MCLAIN, D. R., R. E. BRAINARD, AND J. G. NORTON. 1985. Anomalous warm events in eastern boundary current systems. CalCOFI Rep. 26: 51–64.

METHOT, R., AND J. HIGHTOWER. 1988. Status of the Washington–Oregon–California sablefish stock in 1988. Appendix B in Status of the Pacific Coast groundfish fishery through 1988 and recommended acceptable biological catches for 1989. Pacific Fishery Management Council, Portland, OR.

MURPHY, G. 1965. A solution to the catch equation. J. Fish. Res. Board Can. 22: 191–202.

PALOHEIMO, J. E., AND L. M. DICKIE. 1964. Abundance and fishing success. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 155: 152–163.

PETERMAN, R. M., AND G. STEER. 1981. Relation between sport-fishing catchability coefficients and salmon abundance. Trans. Am. Fish. Soc. 110: 585–593.

POPE, J. G., AND D. J. GARROD. 1975. Sources of error in catch and effort quota regulations with particular reference to variation in the catchability coefficient. Int. Comm. Northwest Atl. Fish. Res. Bull. 11: 17–30.

RADOVICH, J. 1982. Catch-per-unit-of-effort: fact, fiction, or dogma. CalCOFI Rep. 18: 31–34.

SHANNON, L. V., AND S. C. PILLAR. 1986. The Benguela ecosystem. Part III. Plankton. Oceanogr. Mar. Biol. Annu. Rev. 24: 65–170.

SHELTON, P. A., AND M. J. ARMSTRONG. 1983. Variations in parent stock and recruitment of pilchard and anchory populations in the southern Benguela system. *In* G. D. Sharp and J. Csirke [ed.] Proceedings of the Expert Consultation to Examine Changes in Abundance and Species Composition of Neritic Fish Resources, San Jose. Costa Rica, April 1983. FAO Fish. Rep. 291(3): 1113–1132.

WINTERS, G. H., AND J. P. WHEELER. 1985. Interaction between stock area, stock abundance, and catchability coefficient. Can. J. Fish. Aquat. Sci. 42: 989–998.

Ontogeny of Feeding Behavior of First-Feeding Atlantic Salmon (*Salmo salar*)

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Coughlin, D. J. 1991. Ontogeny of feeding behavior of first-feeding Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 48: 1896–1904.

Feeding strikes of Atlantic salmon (*Salmo salar*) alevins preying upon *Daphnia* are described using videorecording of synchronous lateral and antero-ventral views. Based on examination of characteristics such as aiming inaccuracy and capture distance, it is demonstrated that feeding behavior significantly improves during the first 2 wk after initiation of exogenous feeding. With increasing experience, young salmon tend to capture prey more quickly and with greater accuracy. First-feeding alevins use a body-ram feeding mode, relying on their swimming motion to overtake and capture prey. After 7–10 d of feeding, the fish change to a suction feeding mode that effectively uses suction generated by expansion of the orobranchial chamber to pull in prey from a distance. Also, feeding behavior of alevins raised on a commercial salmon feed lags developmentally behind the behavior of fish raised on live food. This lag time is short (2–3 d), indicating that despite reports to the contrary, hatchery-raised fish do not require a long time to learn to capture prey effectively in the wild.

La chasse à la *Daphnia* que font les alevins du saumon de l'Atlantique (*Salmo salar*) est illustrée au moyen d'un enregistrement vidéo synchronisé de plans latéraux et antéro-ventraux. D'après des caractéristiques telles que l'imprécision de la visée et la distance de capture, on démontre que le comportement de prédation s'améliore de façon sensible au cours des deux premières semaines après le début de l'alimentation exogène. Le jeune saumon, de plus en plus expérimenté, capture ses proies plus rapidement et ses mouvements sont plus précis. Les alevins qui commencent à chasser se servent de leur corps comme d'un bélier, se contenant de nager pour rejoindre et capturer leur proie. Au bout de 7 à 10 jours, ils adoptent un mode d'alimentation par succion, c'est-à-dire qu'ils aspirent leur proie en tirant partie de l'effet de succion que produit la dilatation de la cavité orobranchiale. L'évolution comportementale des alevins d'élevage nourris avec des aliments de fabrication commerciale est retardée par comparaison au poisson d'élevage nourri de proies vivantes. Le retard est faible (2 ou 3 jours), ce qui signifie que contrairement à qu'on a dit, le poisson d'élevage n'a pas besoin d'une longue période d'apprentissage pour savoir comment capturer une proie en milieu naturel.

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Young Atlantic salmon (*Salmo salar*) undergo tremendous mortality between the stages of egg deposition and dispersal (0+ parr). Survival of natural populations during this period has often been reported as less than 5% (Meister 1962; Stewart 1963; Côté and Pomerleau 1985). The causes of this mortality are many, including predation, starvation, and abiotic conditions such as temperature, pH, and water quality (Hunter 1981; Bley and Moring 1988). The majority of this mortality occurs when yolk sac alevins change to exogenous feeding as emergent fry and is associated with the difficulties of first-feeding and with intense competition for feeding territories (Ottaway and Clarke 1981). While survival from egg deposition to hatching in both natural and hatchery conditions is usually 70–90%, survival of emergent fry is less than 30% (Bley and Moring 1988) and has been reported as low as 1–5% (Stewart 1963; Danie et al. 1984; MacKenzie and Moring 1988).

Surival in the wild of hatchery-raised alevins correlates with the feeding history of the fish, with differential survival between alevins stocked as unfed and those stocked as fed (Bley and Moring 1988). Stewart (1963) reported survival of stocked, fed fry to the 0+ parr stage as 8.8% whereas for unfed fry, survival was 1.7%. Few other studies have examined survival of unfed versus fed fry in the same river, but Côté and Pomerleau (1985) did report that most of the mortality of stocked, unfed dry occurred in the first 40–80 d after stocking. Also, and possibly more importantly, survival during this early time period was far more variable than during later stages of development. Bley and Moring (1988) speculated that survival of stocked fry in "marginally accepted" rivers is enhanced by feeding fry before release.

The behavioral basis for this difference in survival may stem from the fact that a predator's ability to capture prey increases with experience. Learning is critical to juvenile fish (Marcotte and Browman 1986), and a component of this learning is involved in improving the accuracy of target-oriented feeding behavior, thereby improving feeding success (Drost et al. 1988). During the onset of exogenous feeding, fish are learning to aim, to use suction, and to handle different prey. Examination of the effects of different experiences during the onset of feeding on the resulting prey capture mechanisms reveals the critical factors involved in the learning of feeding behavior.

Many fish are affected by changes in the prey type available to them. In a study on brown trout (*Salmo trutta*), a comparison of survival after 2 yr in a lake was made between fish stocked

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into the lake as hatchery raised versus those which were "pre-stocked" into ponds before being moved to the lake. Pre-stocked fish that had more experience with a variety of prey types in general had less annual mortality and higher growth rates (Hesthagen and Johnsen 1989). Rainbow trout (*Oncorhynchus mykiss*) experience a period of little or no feeding for approximately 4 d when switched from one food type to another (Ware 1971). When larvae of the cichlid *Cichlasoma managuense* that are raised on *Artemia* are switched to feeding on a novel prey, *Daphnia*, capture success (the ratio of successful prey captures to capture attempts) increases with experience (Meyer 1986). Also, these fish adapt more quickly to a new prey type at older ages (Meyer 1988). First-feeding white sturgeon (*Acipenser transmontanus*), however, do not show differences in feeding behavior when switched from one prey type to another, regardless of prior experience with the new prey (Lindberg and Dorosov 1986). However, this study did not examine feeding behavior in great detail.

Feeding behavior of Atlantic salmon differs between hatchery-reared and wild fish (Fenderson et al. 1968; Dickson and MacCrimmon 1982). Feeding in alevins of *S. salar* typically involves the fish staying near the bottom of streams and attacking prey that move by above them (Rimmer and Power 1978; Wankowski 1981). Hatchery-reared alevins tend to station themselves further from the bottom than wild alevins (Dickson and MacCrimmon 1982), but tend to have depressed feeding rates (Fenderson et al. 1968). Marcotte and Browman (1986) speculated that *S. salar* alevins reared in hatcheries on artificial foods may take up to 2 mo to learn to feed on live food. Also, it may take hatchery-reared alevins up to 2 mo after release to develop the diversity of diet found in wild alevins (Sosiak et al. 1979), although they do attack novel prey items readily (Rimmer and Power 1978).

Currently, little is known about the ontogeny of feeding behavior in *S. salar*, particularly with regard to prey capture mechanisms. This study examines the mechanisms of feeding behavior acquisition in *S. salar*. The principal questions are (1) how do Atlantic salmon feeding strikes develop during the first few weeks of feeding? and (2) what effect does prey type have on the feeding behavior and capture success of the emergent fry? Investigation into the development of feeding behavior in Atlantic salmon will contribute to an understanding of the mortality associated with the onset of feeding as well as the differential survival of alevins stocked as unfed versus fed.

Materials and Methods

Unfed *S. salar* alevins, at a stage of approximately 85–90% yolk absorption, were obtained from the Roger Reed Hatchery of the Massachusetts Division of Fisheries and Wildlife, Palmer, Massachusetts, in April 1990. The fish were spawned from sea-run adult Atlantic salmon that were captured from the Connecticut River in June 1989. Fertilization was carried out in early November 1989, and the fish hatched during the last week of February 1990.

The fish were transported to Boston University and maintained there throughout the experiments. Approximately 1000 alevins were sorted roughly into two lots and placed in two 10-gallon (1 U.S. gallon = 3.79 L) aquaria. Water temperature was kept at 10°C, aeration was continuous, and a slow current was generated within each aquarium with an aquarium powerhead. Aquarium water was replaced with aged deionized water two to three times daily, totalling 70% of total tank volume per day.

The alevins were fed twice daily. Half of the alevins were raised on a live food diet, consisting of a 6:1 mixture of *Artemia* and *Daphnia pulex* (live food group). The *Daphnia* were cultured in the laboratory. Cladocerans such as *Daphnia* are a component of the natural diet of wild salmon alevins (Rimmer and Power 1978; Arnemo et al. 1980). Food density for this group was maintained at 200–250 prey·L^{-1}. The other half were raised on a commercially prepared salmon feed, BioProducts BioDiet Starter No. 3 (artificial food group). The density of this food was maintained such that the buildup of food on the bottom of the aquarium was not excessive. The size of prey items in the live food group diet ranged from 0.7 to 1.4 mm, while the pellets of BioDiet ranged from 0.8 to1 .2 mm in size. These size ranges correspond to the size ranges selected by first-feeding *S. salar* alevins (Browman and Marcotte 1986). Throughout this study, alevin survival was greater than 90% for both groups.

Videorecording was carried out with the CritterSpy, a computer-controlled imaging system developed in this laboratory (Strickler 1985). This apparatus consists of two videocameras mounted at right angles to each other, providing front and side views of a filming vessel. Position data from all three dimensions can be derived from the two views. Also, the cameras were mounted on a moving platform that permitted tracking of objects moving within the filming vessel. Image magnification ranged from approximately 15 to 20× (varying with the video monitor in use). Because a darkfield modified-Schlieren optical system was used (see Strickler 1985 for a description), the alevins and prey were visible in profile as dark shapes with crisp white borders. Algae, dust, and other particles greater than 20 μm in size were clearly visible as bright points of light. Electronically shuttered Pulnix TM-845 videocameras were used with Panasonic NV-8500 VCR's and Sony Triniton 13-in. and Panasonic WV-5470 19-in. videomonitors. The filming vessel was lit from above with strong white light from a fiber optics lamp. The Schlieren optical pathway was lit with a He-Ne 1-mW laser-emitting red light of wavelength 632 nm. At no time did the fish appear to respond adversely to the lighting conditions.

On each filming day, the fish were not fed in the morning before filming. For each filming period, 15 alevins were placed in the 5-L filming vessel. Food was added to initiate feeding by the test fish. After a 30-min adjustment period, single prey were added to the vessel. The CritterSpy allowed for tracking individual prey items with the videocameras as they swam or drifted in the currents generated within the vessel by advection with the warmer room air or by the fish themselves. The prey were followed until they were eaten or lost. Feeding strikes were recorded on videotape from both camera views, and only strikes appearing in both views were analyzed.

Development of feeding behavior was examined by filming the feeding of *S. salar* alevins from the live food group. The prey was *D. pulex*. Filming was caried out on days 1, 2, 3, 4, 5, 6, 8, 10, 12, and 15 after first-feeding. Food was introduced to the animals for the first time on day 0; filming was attempted at that time, but the number of actively feeding animals was too low and no results were obtained. On each filming day, six fish were preserved with a 10% formaldehyde solution. Fork lengths of preserved specimens were measured to the nearest 0.01 cm.

To examine the effect of diet on feeding behavior, the feeding behavior of alevins from the artificial food group was compared with that of the live food group. Feeding strikes of alevins from the artificial group feeding on *Daphnia* were filmed on days 8, 10, and 12. Filming of artificial food group alevins was attempted during the first week of feeding (on days 1, 3, and 5) but was not successful because the fish did not feed actively on the live food.

Feeding strikes were analyzed by transferring the 1/2-in. video images to 3/4-in. U-matic videotape. The 3/4-in. tapes could then be reviewed on a field by field basis with a Sony VO-5850 VCR. This permitted temporal resolution of 16.6 ms between images.

The following behaviors were examined: *feeding strike*, a lunge forward by a fish towards a prey while increasing the volume of its buccal and opercular (orobranchial) cavities to effect capture; *capture distance*, the distance from the original prey position when a feeding strike began to the point where it entered the fish's mouth (through suction); *capture time*, the length of time, in blocks of 16.6 ms, between onset of mouth opening and completed capture (when prey entered the mouth); the *perpendicular uptake distance* (Drost 1987), a measure of the accuracy of the alevin's feeding strikes (described further below); and *attack speed*, the speed of the alevin during capture calculated as the distance moved between the video field 33.3 ms before capture to the video field when capture was completed. The length of prey in each feeding strike was also measured.

In general, each feeding strike began with the compression of the orobranchial chamber and the slow opening of the mouth. While still moving towards the prey, the fish would quickly open the mouth and expand the buccal cavity. Prey capture was affected by a combination of lunging at the prey and suction. Prey capture was considered complete when the prey had been taken fully into the fish's mouth.

Other data obtained from the video included the relative timing of compression of the orobranchial chamber and the timing of the opening of the branchiostegal and opercular valves. To determine relative timing, the video field when a feeding strike was initiated (i.e. onset of mouth opening) was determined for each strike. The number of video fields between this point and peak buccal compression was counted; this value could be positive or negative. Peak buccal compression was indicated by adduction of the suspensoria and operculae. Also, the number of video fields after the beginning of a feeding strike and the opening of the opercular valves was counted. Both of these activities were also compared with the time of prey capture.

A perpendicular uptake distance (PUD) was defined as the distance from the centroid of the prey to the length axis of the fish extending through the center of the mouth opening (see Drost 1987). For calculating the PUD of a feeding strike, the centroid of the prey and the position of the length axis were determined on the video field when mouth opening had begun. Working with both views of each feeding strike, horizontal and vertical signed projected perpendicular uptake distances (PPUD's) were measured. This was a modification of the definition of PUD used by Drost (1987). Drost measured the distance from the centroid of the prey to the length axis passing through the center of the mouth at the point of peak gape. Because video was used in this study and not high-speed cinematography, it was impossible to consistently determine the point of peak gape.

A positive PPUD value denoted a prey position either above or to the right of the length axis. The pythagorean sum of vertical and left–right PPUD's resulted in PUD. The expected mean value of PUD is zero, meaning that the fish aim straight at the prey. The mean values of each PPUD were termed the aiming target, and the standard deviations were termed the aiming inaccuracy of a group of strikes (Drost 1987). Parallel aiming is more difficult to quantify because it is difficult to judge exactly where the fish was aiming along the axis extending straight ahead from its mouth. Instead, parallel aiming is discussed in qualitative terms.

Results

Feeding Behavior of Salmon Alevins

The two groups of alevins showed similar growth curves during the 2 wk of this study. The fish obtained from the hatchery had mean ($\pm$ SD) fork lengths of 26.8 $\pm$ 1.1 mm (n = 10). The live food group ended the study with mean fork length of 29.3 $\pm$ 0.5 mm (n = 15) and the artificial food group finished the study with a mean fork length of 29.2 $\pm$ 0.7 mm (n = 15).

During a filming session, fish spent the majority of their time on the bottom, propped on their pectoral fins and oriented into the slow current. As a food item drifted in the current, a fish would visually fix on the prey, swim quickly towards it, strike at it, and return to the bottom of the vessel. This sequence of feeding behavior was similar to the direct interception of drifting prey by alevins previously described (Wankowski 1981; Browman and Marcotte 1986).

As the fish closed in on the prey, it initiated a feeding strike, typically at a distance of 5.0 mm from the prey. The duration of the feeding strike was usually less than 90 ms from opening of the mouth to closure after capture. At the beginning of a feeding strike, prey were typically located 0.3 mm above the length axis of the fish, indicating that the fish tended to aim below the centroid of the prey. Sometimes, the fish would turn slightly upward during the final video field before capture to correct for aiming low. Normally, however, the prey entered the mouth through the upper half of the mouth opening. In the horizontal plane, the larvae did not bend to a great degree; lateral excursions after the onset of mouth opening were minimal (Fig. 1). Attack speed remained around 8 $cm \cdot s^{-1}$ or 3 body lengths$\cdot s^{-1}$ for all groups of fish examined.

Ontogeny of Feeding Behavior

For this section, results are only presented for salmon alevins from the natural, live food group.

During the first few days of feeding, the fish often pushed the prey away from its original position before capturing it, resulting in a negative capture distance and sometimes in a miss. This pushing away was caused either by a failure of the fish to open its mouth wide enough and fast enough as it lunged at the prey or by the water entrained by the fish's movement. Also, at this early stage of feeding the fish tended to aim poorly, often having to correct for their error during a lunge (Fig. 2). During the last few days of this study, the alevins captured their food from a distance through the effective use of suction. Also, the suction flow was highly directional (Fig. 3). The aiming of their lunges improved and they tended to open their mouths wider and more quickly (Fig. 2).

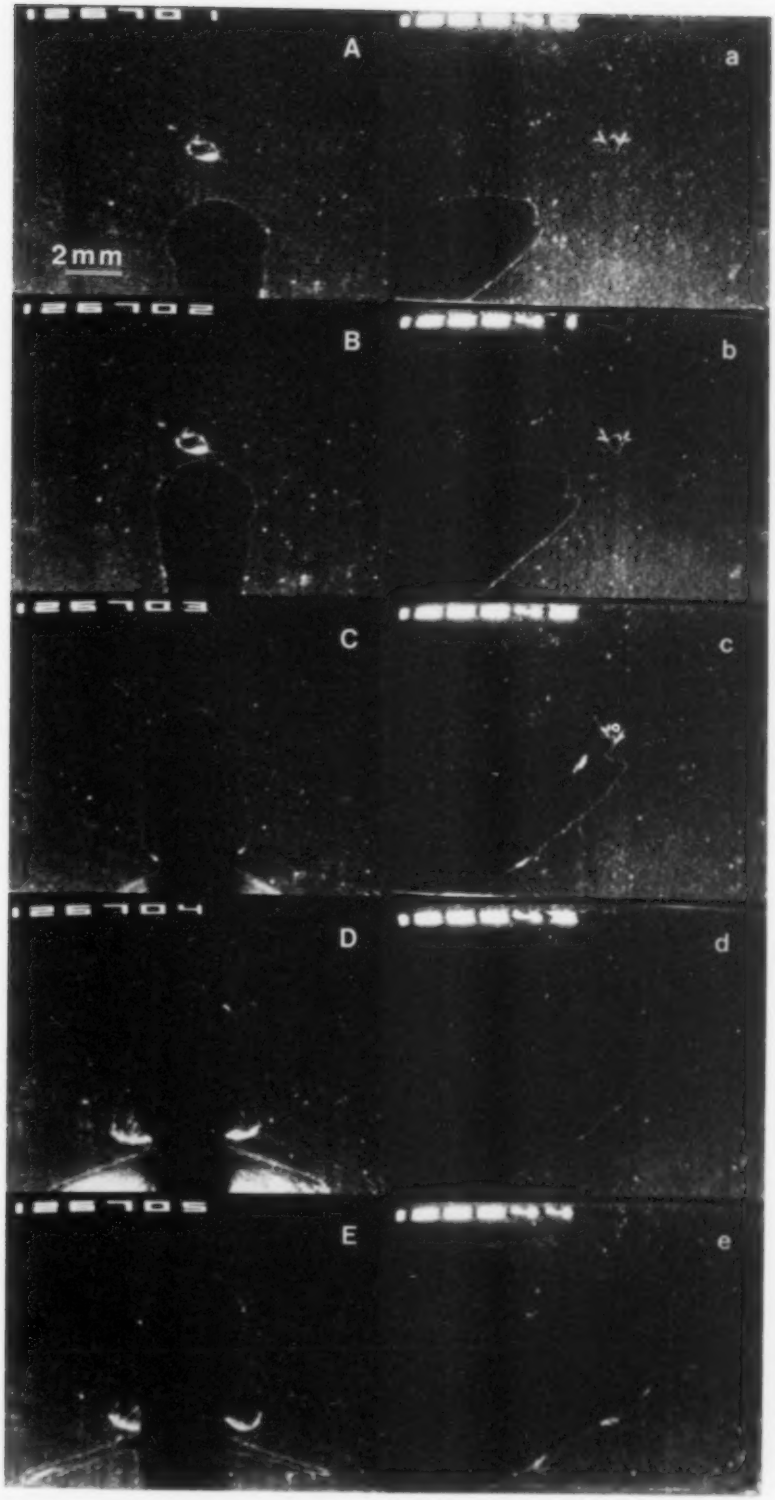

FIG. 1. (*Concluded next page*)

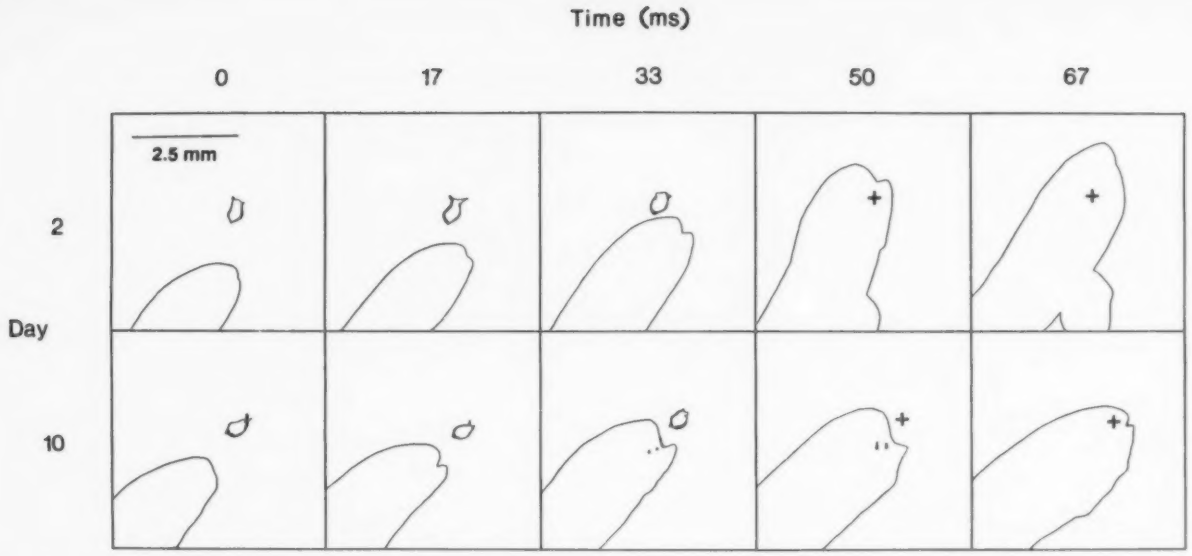

FIG. 2. Prey capture by *S. salar* alevins feeding on *Daphnia* showing a lateral view of feeding strikes as recorded on videotape. Shown are representative strikes of feeding at days 2 and 10 after the initiation of feeding. Plus signs indicate the prey's original position on panels after the prey has been captured. At day 2, the fish tend to aim low and overrun the prey position after capture. By day 10, suction as the mechanism of prey capture is apparent.

For the live food group, data from the following pairings were pooled because of a low number of observations: days 1 and 2, days 3 and 4, and days 5 and 6. Results are therefore reported for days 1–2, 3–4, 5–6, 8, 10, 12, and 15 for the fish raised on live food. Results from the artificial food group are discussed in the next section.

During the first 2 wk of feeding by *S. salar* alevins, there was a significant trend towards capture of prey from progressively farther away through the improved use of suction (Fig. 4; Kruskal-Wallis nonparametric analysis of variance, $p < 0.001$). From a nonparametric Tukey-type multiple comparison (Zar 1984), it was shown that capture distances were each significantly lower on days 1–2, 3–4, and 5–6 than on days 12 and 15 (1–2 versus 12 and 15, $p < 0.001$; 3–4 and 5–6 versus 15, $p < 0.002$; 3–4 and 5–6 versus 12, $p < 0.005$).

At the same time, there was a significant trend towards greater consistency in behavior as fish got older. The variance of capture distance, as measured by the standard deviation, significantly declined with time (Fig. 4; Barlett test of homogeneity of variances, $p < 0.001$). A parametric Tukey-type multiple comparison (Zar 1984) showed that the variances of days 1–2, 3–4, 5–6, and 8 were each significantly higher than on days 10, 12, and 15, with the exception of day 3–4 versus 12 (1–2, 3–4, 5–6, and 8 versus 10 and 15, $p < 0.001$; 1–2 versus 12, $p < 0.005$; 8 versus 12, $p < 0.02$; 5–6 versus 12, $p < 0.05$).

Aiming improved over the course of 2 wk as well. There was a significant trend towards lower projected uptake distances with increasing age (one-tailed ANOVA, $p < 0.05$). A multiple comparison Tukey-test, however, failed to show significant differences between any two days. Additionally, although there was an apparent trend towards a reduction in variance (Fig. 5), a Bartlett's test of homogeneity of variance showed no significant differences in variance of PUD with respect to age ($p > 0.25$). The improvement in aiming was apparent in the increase in capture success. The combined capture success for days 1–6 was 87% (51 successful captures/59 capture attempts), while capture success increased to 97% (70/72) for days 8–15. The respective size ranges for these two time periods were 27.0–28.5 and 28.2–29.3 mm.

An examination of the horizontal PPUD's showed that the fish's horizontal aiming target was straight at the prey; the horizontal PPUD did not significantly differ from zero at any age (Single sample *t*-tests). Vertical PPUD's, however, were consistently greater than zero (Table 1), indicating that the fish's vertical aiming target was below the prey. On days 3–4, 10, 12, and 15, the vertical PPUD was significantly greater than zero (*t*-test, $p < 0.05$).

FIG. 1. Prey capture by a *S. salar* alevin at 12 d after the initiation of feeding on *Daphnia*. Pictures were transferred from the videotape recording of a single feeding strike. (A–E) Antero-ventral views; (a–e) lateral views. Time between pictures is 16.6 ms. The prey is located directly in front of the fish in Fig. 1A, 1a, 1B, and 1b, and it was captured in approximately 34 ms. In Fig. 1C and 1c, the use of suction is apparent. In Fig. 1C, the operculae are being abducted, and in Fig. 1c, the prey is entering the fish's mouth; a small white circle indicates its initial position at the beginning of the feeding strike (the reference frame shifts slightly during the strike). In Fig. 1D and 1E, the fish repositions its pectoral fins to stop its forward motion.

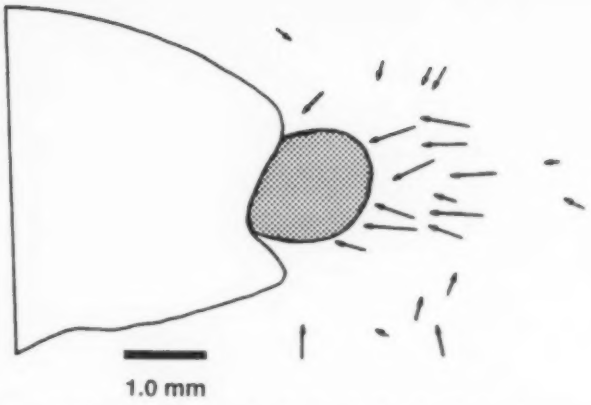

FIG. 3. Water movement during feeding by *S. salar* alevins. The outline is a lateral view of an alevin's head when prey capture is completed. The shaded zone is the capture area from which water has been sucked into the fish's mouth during the previous 16.6 ms; the prey, a *Daphnia*, was located at the center of this area. The arrows are the paths of particles in the surrounding water; arrow length indicates relative particle velocity. All movement is relative to an earth-bound frame. This figure combines particle tracings from five feeding strikes on day 12.

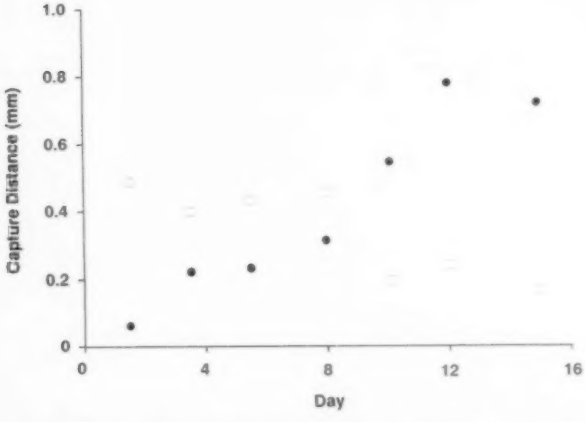

FIG. 4. Capture distance from *S. salar* alevins feeding on *Daphnia*. Capture distance (solid circles) and the standard deviation of capture distance (open squares) are plotted as functions of the number of days after the initiation of feeding. Capture distance increases with a slope of 0.05 mm·d^{-1} (R^2 = 0.90); the standard deviation decreases with a slope of −0.02 mm·d^{-1} (R^2 = 0.75).

Prey capture during a feeding strike tended to take less time as the alevins got older. During the first few days of feeding by *S. salar* young, almost all recorded feeding strikes required 33–66 ms. By day 10, prey capture in the majority of feeding strikes was completed in 17–33 ms (Fig. 6).

The more effective and coordinated use of suction was seen through an analysis of movement of the opercular bones. During the early stages of feeding the fish compressed their mouth cavities through adduction of the suspensoria and operculae 17 ms or less before prey capture (9 of 14 observations from day 1–2), while the mouth was already starting to open. Older alevins compressed their buccal cavities 33–66 ms before prey

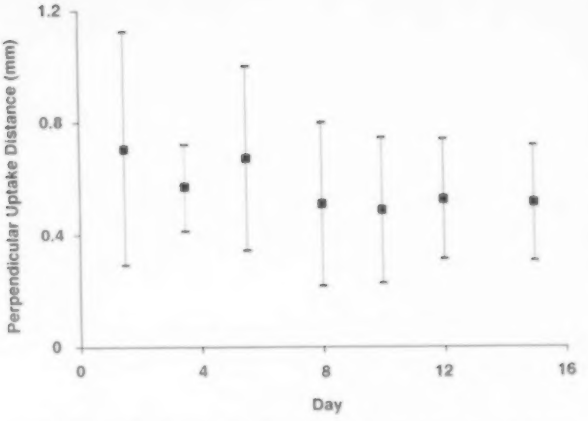

FIG. 5. Perpendicular uptake distance (PUD), a measure of aiming inaccuracy, from *S. salar* alevins feeding on *Daphnia*. Vertical bars represent the standard deviation; days are the number of days after the initiation of feeding.

TABLE 1. Vertical and horizontal prey uptake distances (PUD) from *S. salar* alevins, measured as the distance from the centroid of the prey to the length axis of the fish (Drost 1987). Positive values designate prey positions above or to the right of the fish. Day is number of days after initiation of feeding. Values are mean ± SD. *Significant difference from zero ($p < 0.05$).

Day	Vertical	Horizontal	*N*
1–2	0.36 ± 0.61	0.12 ± 0.39	12
3–4	0.39 ± 0.20*	−0.04 ± 0.39	9
5–6	0.51 ± 0.37	−0.05 ± 0.40	11
8	0.18 ± 0.28	0.25 ± 0.41	10
10	0.31 ± 0.16*	0.02 ± 0.43	11
12	0.31 ± 0.09*	0.22 ± 0.41	7
15	0.30 ± 0.15*	−0.08 ± 0.43	11

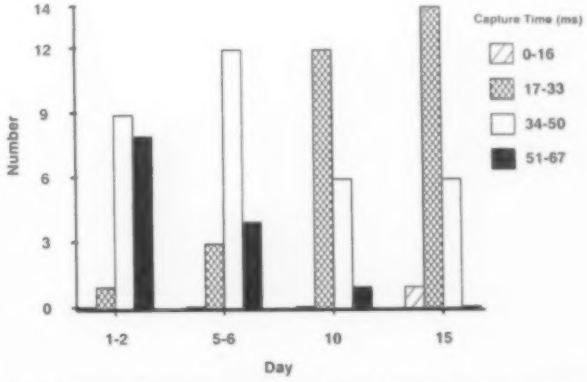

FIG. 6. Capture time, the time from initiation of feeding strike at mouth opening to completed prey capture, from *S. salar* alevins feeding on *Daphnia*. The distribution of capture time in blocks of 16.6 ms is reported for days 1–2, 5–6, 10, and 15 after initiation of feeding by alevins.

capture, before mouth opening had begun (8 of 13 observations from day 15). Suction generated by expansion of the orobranchial chamber through simultaneous hyoid depression and

TABLE 2. Comparison of feeding behavior of *S. salar* alevins raised on live food with that of alevins raised on a commercially prepared diet. Day indicates number of days after initiation of feeding. Values are means ± SD, and the units are mm. Capture distance is the distance from the prey's original position to the point of its capture (positive values indicate suction). PUD is the perpendicular uptake distance, a measure of perpendicular aiming (zero indicates aiming straight at the prey). Speed ($mm \cdot s^{-1}$) is of the fish at the point of capture. Success is the percentage of successful feeding strike attempts. *Significant difference between groups ($p < 0.05$).

	Day 8		Day 12	
Group	Live	Artificial	Live	Artificial
N	14	16	15	13
Fork length	28.2 ± 0.6	28.0 ± 0.8	29.1 ± 0.8	28.7 ± 0.5
Capture distance	0.47 ± 0.70	0.32 ± 0.78	1.18 ± 0.38	0.41 ± 0.79*
PUD	0.51 ± 0.29	0.50 ± 0.25	0.53 ± 0.21	0.41 ± 0.13
Attack speed	78.7 ± 29.4	91.5 ± 19.5	68.1 ± 13.3	65.2 ± 17.7
Capture success	93	100	93	92

abduction of the suspensoria was used to complete prey capture. As in other salmonids, opercular dilation occurred late in the feeding sequence, after the movement of the hyoid and suspensoria (Lauder and Liem 1980).

In the younger alevins, opening of the opercular valves occurred in the same video field as prey capture was completed (13 of 14 observations). Older alevins waited 17 ms or more after prey capture before opening their opercular valves (11 of 13 observations). This shift may simply have resulted because the time from the opening of the mouth at the beginning of a feeding strike to the opening of the opercular valves did not change during the first weeks of feeding. While older fish captured prey more quickly, the duration of the feeding strike remained the same. So, opening of the opercular valves occurred after prey capture instead of at the same time (Fig. 1).

Effect of Food Type on Feeding Behavior

In general, feeding behavior of the artificial food group fish was developmentally retarded in comparison with fish of the same age from the live food group; fish raised on artificial food developed feeding behavior at a slower rate than fish raised on living food. The data reported here are for days 8 and 12. There were insufficient data to extend the analysis to day 10.

The main component of feeding behavior that differed between the artificial and live food groups was capture distance (Table 2). On both days, capture distance was greater for alevins raised on live food than for those raised on artificial food, but this difference was only significant for day 12 (Mann-Whitney *U*-test, $p < 0.005$). The variance of capture distance was less for the live food group than the artificial food group at each age, but, again, this difference was significant only for 12 (F-test, $p < 0.01$).

The only other notable difference in behavior was in the timing of opercular movements. At day 8, the live food group alevins tended to compress their mouth cavities sooner relative to mouth opening than did alevins from the artificial food group. Live food group fish usually had maximum compression at the point of mouth opening, while artificial food group alevins more often opened their mouths before completing compression of the mouth. The behavior of the artificial food group alevins at this age was similar to that of slightly younger live food group alevins. By day 12, there were no discernable differences in opercular movements.

Attack speed, aiming inaccuracy, and capture success were similar for alevins from both groups at each age (Table 2). Capture success was high for both groups on both days, and attack speed declined from day 8 to day 12 in both groups.

Discussion

Feeding by *S. salar* alevins combines swimming quickly towards prey and suction to capture food. During the first 2 wk of feeding by alevins, the reliance of the fish on swimming diminishes as the use of suction improves. There was a gradual transition from a lunging, ram-feeding prey capture, where prey were captured by the fish overrunning the prey's position, to a suction feeding mode, where prey were sucked into the mouth of the fish while the fish were still some distance from the prey's original position.

Atlantic salmon alevins use a "low-τ suction with swimming" feeding mechanism, particularly during the first few days of feeding. Similar to the behavior described here for salmon alevins, low-τ or velocity suction involves early opening of the opercular valves, relatively high importance of swimming to prey capture, and highly directed flow during suction (Osse and Muller 1980; Muller and Osse 1984; Muller and Van Leeuwen 1985). Also, the streamlined body shape, primitive jaw structure, and taxonomic classification (protacanthopterygian) agree with the morphological criteria described by Muller and Osse (1984) for this type of feeding mechanism. Because swimming was less important to prey capture and there was a delay in the opening of the opercular valves, feeding of older alevins was not as extreme an example of velocity suction.

The directional nature of suction prey capture in salmon alevins differs from the flow patterns generated by fish using volume suction. In the sunfish (*Lepomis gibbosus*), flow tends to come from all directions towards the mouth during suction (Lauder and Clark 1984). This undirected flow is more common in fish using volume suction (Muller and Osse 1984).

The feeding behavior of young salmon alevins is generally inefficient. Several characteristics of their feeding behavior are counterproductive to prey capture. First, alevins feeding for the first time tend to open their mouths before completing compression of the buccal and opercular cavities. This theoretically causes a pulse of water to be directed out of the mouth, ahead of the fish as compression is completed, pushing the prey away. Also, young salmon do not have a flat, round oral opening during suction. Instead, their mouths appear rounded from the

ventral view and notched when viewed laterally (Fig. 1). The notch is visible even at peak gape, not just when the mouth is closed. The curvature of the mouth means the lateral portions of the orifice do not point directly forward, but slightly laterally. As has been reported, the curvature and the notch allow water to flow in from the sides during suction, reducing the directionality of suction and decreasing the volume of water sucked from directly ahead of the fish (O'Brien 1979; Drost and van den Boogaart 1986). Lastly the fish's ability to suck is reduced by the early opening of the opercular valves in feeding strikes from the first few days of feeding (Muller and Osse 1984), although this is a point of contention among researchers today. The early opening of the valve probably does permit unidirectional flow caudad from the mouth, reducing the effects of the fish's entrained water mass (Lauder et al. 1986).

First-feeding salmon were poor at both perpendicular and parallel aiming. They showed high variability in PUD, particularly in the vertical component; sometimes the PUD was great enough to cause a miss. In regard to parallel aiming, they sometimes initiated feeding strikes earlier or later than would be ideal. This caused a miss because suction was completed before they reached the prey or because they did not open their mouths fast enough and pushed the prey away in the entrained water mass that moved around the fish as it swam.

When the swimming component of prey capture is strong relative to suction, as in first-feeding *S. salar*, the prey should theoretically escape in a direction perpendicular to the movement of the fish (Drost et al. 1988). A prey will best avoid a swimming predator if it moves out of the predator's path. Therefore, perpendicular aiming should be more important to capture success than parallel aiming. In this study, however, most misses by first-feeding alevins were caused by parallel aiming errors. Capture success did not depend on perpendicular aiming, but on the coordination of the timing of feeding strikes. At first-feeding, the fish were already able to aim sufficiently well in left–right and up–down dimensions; to capture food they had to coordinate the timing of the feeding strikes as they approached the prey.

Among the older alevins that were more effectively using suction, coordination aiming steadily improved. One sign of this was the significant decrease in the variability of capture distance as the fish consistently timed the feeding strike. With this improvement in aiming at the same time that suction was improving, capture success increased. These two factors worked together because improved suction allowed the fish to suck in the water directly in front of it as it approached a prey, so the prey did not get pushed away. As demonstrated by Drenner et al. (1978) in a study of the evasive abilities of different prey types, *Daphnia* are a relatively easy prey to capture. Once the salmon had adequately developed its feeding behavior, it captured cladoceran prey with high success (100% for day 15). With increasing age, the suction feeding of salmon alevins became similar to the feeding of sunfish (*Lepomis*) (Lauder 1980).

When feeding on highly evasive prey such as copepods (Drenner et al. 1978), success should be much lower. In my study, a few strikes were recorded of alevins feeding on cyclopoid copepods; all were misses. In each case, the evasive responses of the copepods were successful. Salmon show a strong preference for copepods, however, when selecting between cladocerans and copeods over a wide range of prey densities (Browman and Marcotte 1986, 1987). Future work should examine how capture success of alevins feeding on evasive prey varies with developmental state. Since capture success would probably be much lower for these prey, alevins may have to develop suction feeding more rapidly to obtain a sufficient amount of food each day.

Unlike larval carp (*Cyprinus carpio*) and larval and juvenile northern pike (*Esox lucius*), which tend to aim above their prey (Drost 1987), *S. salar* tends to aim "low." Aiming low may occur because the fish regularly approach prey from below. Also, aiming low compensates for reorientation in head position that occurs when the epaxial muscles are used to widen the gape of the mouth. The epaxial muscles elevate the neurocranium, bending the head up and opening the mouth. Since neurocranium elevation is an important component of feeding by salmonids (Lauder and Liem 1980), the alevins may originally aim "low," but their aim is the correct compensation that allows them to capture prey straight on. If this is true, then one would expect the opposite result in *E. lucius* because pike have been shown to aim above the prey. A comparative study of the feeding behavior of *Esox* and *Salmo* might reveal other more substantial differences.

Many studies have reported that hatchery-raised fish differ from their wild counterparts. Reportedly, hatchery fish have a smaller repertoire of search images (Dickson and MacCrimmon 1982) and a less diverse diet (Sosiak et al. 1979), swim at different heights in a stream (Fenderson et al. 1968), and experience different survival rates (Bley and Moring 1988). This study, however, indicates that hatchery-raised and wild salmon do not differ dramatically with respect to the mechanism of prey capture. The live food group alevins were raised on a mixed diet of *Artemia* and *Daphnia* and here represent wild animals, while the artificial food group alevins were raised on a common hatchery diet. While there does appear to be a lag time in development of suction feeding for *S. salar* raised on an artificial diet versus a more natural diet, this lag time is on the order of 1 or 2 d and not 2 mo as has been suggested for other characteristics of feeding behavior (e.g. Marcotte and Browman 1986).

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References

ARNEMO, R. C., C. PUKE, AND N. G. STEFFNER. 1980. Feeding during the first weeks of young salmon in a pond. Arch. Hydrobiol. 89: 265–273.

BLEY, P. W., AND J. R. MORING. 1988. Freshwater and ocean survival of Atlantic salmon and steelhead: a synopsis. U.S. Fish Wildl. Serv. Biol. Rep. 88(9): 22 p.

BROWMAN, H. I., AND B. M. MARCOTTE. 1986. Diurnal feeding and prey size selection in Atlantic salmon, *Salmo salar*, alevins. Dev. Environ. Biol. Fishes 7: 269–284.

1987. The effect of zooplankton abundance on feeding behaviour and prey size selection in Atlantic salmon, *Salmo salar*, alevins. Holarct. Ecol. 10: 163–170.

CÔTÉ, Y., AND C. POMERLEAU. 1985. Survie et dispersion d'alevins de saumon Atlantique (*Salmo salar*) ensemences en milieu naturel. Naturaliste can. (Rev. Ecol. Syst.) 112: 549–557.

DANIE, D. S., J. G. TRIAL, AND J. G. STANLEY. 1984. Species profiles: life histories and environmental requirements of coastal fishes and inverte-

brates (North Atlantic) — Atlantic salmon. U.S. Fish Wildl. Serv. FWS/OBS-82/11.22 and U.S. Army Corps Eng. TR EL-82-4: 19 p.

DICKSON, T. A., AND H. R. MACCRIMMON. 1982. Influence of hatchery experience on growth and behavior of juvenile Atlantic salmon (*Salmo salar*) within allopatric and sympatric stream populations. Can. J. Fish. Aquat. Sci. 39: 1453–1458.

DRENNER, R. W., J. R. STRICKLER, AND W. J. O'BRIEN. 1978. Capture probability and the role of zooplankton escape in selective feeding of planktivorous fish. J. Fish. Res. Board Can. 35: 1370–1373.

DROST, M. R. 1987. Relation between aiming and catch success in larval fishes. Can. J. Fish. Aquat. Sci. 44: 304–315.

DROST, M. R., J. W. M. OSSE, AND M. MULLER. 1988. Prey capture by fish larvae, water flow patterns and the effect of escape movements of prey. Neth. J. Zool. 38(1): 23–45.

DROST, M. R., AND J. G. M. VAN DEN BOOGAART. 1986. The energetics of feeding strikes in larval carp, *Cyprinus carpio*. J. Fish Biol. 29: 371–379.

FENDERSON, O. C., W. H. EVERHART, AND K. M. MUTH. 1968. Comparative agonistic and feeding behavior of hatchery-reared and wild salmon in aquaria. J. Fish. Res. Board Can. 25: 1–14.

HESTHAGEN, T., AND B. O. JOHNSEN. 1989. Lake survival of hatchery and prestocked pond brown trout, *Salmo trutta* L. Aquacult. Fish. Manage. 20: 91–95.

HUNTER, J. R. 1981. Feeding ecology and predation of marine fish larvae, p. 33–77. *In* R Lasker [ed.] Marine fish larvae. University of Washington Press, Seattle, WA.

LAUDER, G. V. 1980. The suction feeding mechanism in sunfishes (*Lepomis*): an experimental analysis. J. Exp. Biol. 88: 49–72.

LAUDER, G. V., AND B. C. CLARK. 1984. Water flow patterns during prey capture by teleost fishes. J. Exp. Biol. 113: 143–150.

LAUDER, G. V., AND K. F. LIEM. 1980. The feeding mechanism and cephalic myology of *Salvelinus fontinalis*: form, function, and evolutionary significance, p. 365–390. *In* Eugene K. Balon [ed.] Charrs, salmonid fishes of the genus *Salvelinus*. Dr. W. Junk bv Publishers, The Hague, The Netherlands.

LAUDER, G. V., P. C. WAINWRIGHT, AND E. FINDEIS. 1986. Physiological mechanisms of aquatic prey capture in sunfishes: functional determinants of buccal pressure changes. Comp. Biochem. Physiol. 84A(4): 729–734.

LINDBERG, J. C., AND S. I. DOROSOV. 1986. Effect of diet switch between natural and prepared foods on growth and survival of white sturgeon juveniles. Trans. Am. Fish. Soc. 115: 166–171.

MACKENZIE, C., AND J. R. MORING. 1988. Estimating survival of Atlantic salmon during the intragravel period. N. Am. J. Fish. Manage. 8: 45–49.

MARCOTTE, B. M., AND H. I. BROWMAN. 1986. Foraging behaviour in fishes: perspectives on variance. Environ. Biol. Fishes 16: 25–33.

MEISTER, A. L. 1962. Atlantic salmon production in Cove Brook, Maine. Trans. Am. Fish. Soc. 91: 208–212.

MEYER, A. 1986. Changes in behavior with increasing experience with a novel prey in fry of the Central American cichlid, *Cichlasoma managuense* (Teleostei: Cichlidae). Behaviour 98: 145–167.

1988. Influence of age and size on the response to novel prey by fry of the cichlid fish *Cichlasoma managuense* (Pisces, Cichlidae). Ethology 78: 199–208.

MULLER, M., AND J. W. M. OSSE. 1984. Hydrodynamics of suction feeding in fish. Trans. Zool. Soc. Lond. 37: 51–135.

MULLER, M., AND J. L. VAN LEEUWEN. 1985. The flow in front of the mouth of a prey-capturing fish. Fortschr. Zool. 30: 223–227.

O'BRIEN, W. J. 1979. The predator–prey interaction of planktivorous fish and zooplankton. Am. Sci. 67(5): 572–581.

OSSE, J. W. M., AND M. MULLER. 1980. A model of suction feeding in teleostean fishes with some implications for ventilation, p. 335–351. *In* M. A. Ali [ed.] Environmental physiology of fishes. NATO-ASI. Series A. Life science. Plenum Publishing Co., New York, NY.

OTTAWAY, E. M., AND A. CLARKE. 1981. A preliminary investigation into the vulnerability of young trout (*Salmo trutta* L.) and Atlantic salmon (*S. salar* L.) to downstream displacement by high water velocities. J. Fish Biol. 19: 135–145.

RIMMER, D. M., AND G. POWER. 1978. Feeding response of Atlantic salmon (*Salmo salar*) alevins in flowing and still water. J. Fish. Res. Board Can. 35: 329–332.

SOSIAK, A. J., R. G. KANDALL, AND J. A. MCKENZIE. 1979. Feeding by hatchery reared and wild Atlantic salmon (*Salmo salar*) parr in streams. J. Fish. Res. Board Can. 36: 1408–1412.

STEWART, K. 1963. Investigations into migratory fish propagation in the area of the Lancashire River Board. Barber, Lancaster, England. 80 p.

STRICKLER, J. R. 1985. Feeding currents in calanoid copepods: two new hypotheses. Symp. Soc. Exp. Biol. 39: 459–485.

WANKOWSKI, J. W. J. 1981. Behavioural aspects of predation by juvenile Atlantic salmon (*Salmo salar* L.) on particulate, drifting prey. Anim. Behav. 29: 557–571.

WARE, D. M. 1971. Predation by rainbow trout (*Salmo gairdneri*): the effect of experience. J. Fish. Res. Board Can. 28: 1847–1852.

ZAR, J. H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall, Englewood Cliffs, NJ. 718 p.

Some Aspects of Ascorbate Metabolism in Developing Embryos of the Brine Shrimp (*Artemia salina*)

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Dabrowski, K. 1991. Some aspects of ascorbate metabolism in developing embryos of the brine shrimp (*Artemia salina*). Can. J. Fish. Aquat. Sci. 48: 1905–1908.

Cysts of the brine shrimp (*Artemia salina*) during various hydration states were analyzed for ascorbate concentration. At a state of cellular dehydration and metabolic arrest, ascorbate is stored in the form of stable ascorbic sulfate. Cysts from six populations in North America, France, Australia, and China differed considerably in ascorbic sulfate concentration (162.4–428.5 μg·g dry matter^{-1}, expressed as ascorbic acid). Embryos incubated in NaCl solution started to hydrolyze the stable form and released free ascorbic acid. Major transfer of the stable sulfated form to active ascorbic acid (on an equimolar basis) also took place at hatching. Activity of ascorbic sulfate sulfohydrolase was analyzed by two methods and peak activity after hatching corresponds to an increase of free ascorbic acid. However, decapsulation of cysts does not lead to ascorbic sulfate hydrolysis, and therefore raises the question of vitamin C availability for larval fish fed decapsulated *Artemia*.

On a dosé l'ascorbate dans des kystes d'artémia (*Artemia salina*) hydratés à divers degrés. À l'état de déshydratation cellulaire et d'arrêt métabolique, l'ascorbate est stocké sous une forme stable, soit en sulfate ascorbique. On a constaté que la concentration de sulfate ascorbique diffère considérablement dans les kystes de six populations d'Amérique du Nord, de France, d'Australie et de Chine (162,4 à 428,5 μg·g de matière sèche^{-1}, sous forme d'acide ascorbique). Les embryons incubés dans une solution de NaCl ont commencé à hydrolyser la forme stable et à rejeter de l'acide ascorbique libre. On a aussi observé une importante transformation de la forme sulfatée stable en acide ascorbique actif (équimolarité) à l'éclosion. Par ailleurs, on a évalué l'activité de la sulfohydrolase par deux méthodes : l'activité maximale après l'éclosion correspond à l'augmentation de l'acide ascorbique libre. Toutefois, la décapsulation des kystes ne détermine pas l'hydrolyse du sulfate ascorbique, ce qui soulève la question de la disponibilité de la vitamine C pour les larves de poisson nourries avec d'artémies.

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Dry cysts of the brine shrimp (*Artemia salina*) can withstand temperatures of 103°C up to 1.5 h and for several hours as high as 66°C. Hydrated, developing cysts are less tolerant, and 50% of embryos are killed by a temperature of 49°C for 1 h (Miller and McLennan 1988). Such a prolonged heat shock would normally destory intracellular macromolecular structures of eukaryotic organisms including degradation of ascorbic acid. Although the inability of crustaceans to synthesize ascorbic acid is controversial in the literature (Guary and Guary 1975; Desjardin et al. 1985), the remarkable resistance of ascorbic acid in metabolically inactive cysts of brine shrimp is known and explained by the presence of a unique sulfur derivative of ascorbic acid, ascorbic acid-2-sulfate (AS) (Mead and Finamore 1969; Bond et al. 1972; Golub and Finamore 1972).

AS has been claimed to be involved in the sulfation of macromolecules in animals and man (Baker et al. 1971; Mumma and Verlangieri 1972); however, Dabrowski et al. (1990b) reported that AS is rapidly eliminated in fish. Hornig (1975) has shown that AS oral or parenteral administration resulted in urinary (30–60% of the dose) and fecal elimination (30% of the dose) within the first 24 h. Therefore, the bound, sulfated ascorbate represents a relatively small part of ascorbate metabolism in vertebrates. However, it represents the exclusive, stored form of vitamin C in resting eggs of brine shrimp (Dabrowski and Hinterleitner 1989). Still, little is known about the mechanism whereby cyst hydration activates ascorbic sulfate hydrolysis in *Artemia* embryos.

Freshly hatched *Artemia* nauplii are used as a larval fish food, and an alternative, decapsulated cysts, has been tested with good success on African catfish (*Clarias goriepinus*) (Verreth et al. 1987) and common carp (*Cyprinus carpio*) (Vanhaecke et al. 1990). Therefore, I studied the changes of AS/ascorbate relationship in decapsulated cysts and evaluated whole cysts from different geographical strains of brine shrimp with respect to their potential nutritional value and the primary steps in the hydration-activated sequence of events leading to AS desulfation.

Materials and Methods

Experiments

Whole *Artemia* cysts used in this study originated from San Francisco Bay and were purchased from San Francisco Bay Brand, Inc. Cysts were incubated in standard hatching conditions (sea salt, 25 ppt. 25°C) at 2 g cyst·L continuously aerated water^{-1}. The set of three 3-L conical flasks comprised a series. Aeration was not stopped during periodic (2–3 h apart) sampling until mass hatching occurred. After hatching, nauplii were separated from cysts for analysis.

Cysts that were decapsulated were obtained from P. Sorgeloos and Ph. Leger, Artemia Reference Center, Ghent,

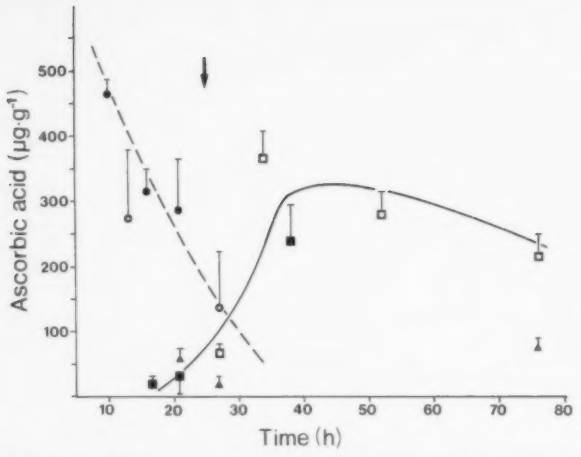

FIG. 1. Ascorbate concentrations in *Artemia* cysts and embryos (mean ± SD). Arrow indicates mass hatching. Two series of incubations are shown as open and closed symbols. ○, ● = AS; □, ■ = total AA; △ = DHA. Broken and solid lines were fitted to represent the average concentration of AS and AA, respectively.

Belgium, and kept refrigerated (4°C) before analysis. More details on the origin of decapsulated cysts are provided in Table 2. Cyst preparation was performed according to Sorgeloos et al. (1983).

Ascorbate Determination and Enzyme Assays

Frozen organisms were homogenized in the cold extraction solution and total ascorbic acid (AA), dehydroascorbic acid (DHA), and AS were analyzed according to the methods already described (Dabrowski and Hinterleitner 1989; Dabrowski et al. 1990b).

Ascorbic sulfate sulfohydrolase (ASS-ase) was measured according to two procedures (Dabrowski et al. 1990b). Frozen organisms were homogenized in cold 50 mM Tris–HCl buffer, pH 7.5, centrifuged at 11 000 × *g* for 3 min and dialyzed for 24 h in two changes of Tris buffer at 4°C. Direct assay measured amount of released ascorbate in the mixture containing 0.1 M sodium acetate (pH 5.5), AS (20 mM), albumin (0.02%), and enzyme. The reaction was carried out at 28°C for 3 h and stopped by adding 200 µL of the reaction mixture to 800 µL of TCA–PCA–EDTA solution (see AA determination in Dabrowski and Hinterleitner 1989). Indirect assay of ASS-ase consisted of the reduction of 2,6-dichloroindophenol (DCIP) by AA released by enzymatic hydrolysis of AS (Carlson et al. 1977). Twenty microlitres of the enzyme solution was added to 430 µL of a 0.2 mM DCIP solution in 0.1 M sodium acetate buffer (pH 5.5) and incubated 15 min at room temperature. The sample was centrifuged at 11 000 × *g* for 3 min and 400 µL of supernatant transferred to a spectrophotometry cuvete. Decrease of absorbance at 570 nm was measured during 10 min following addition of 45 µL of 100 mM AS and compared with blanks without enzyme or without substrate run simultaneously. The decrease in absorbance when standards of known amount of ascorbate were assayed in the same condition allowed calculation of liberated ascorbate.

Results

At the onset of hatching of *Artemia*, AS was present in nauplii, although small contamination by cysts cannot be excluded (Fig. 1). In nauplii separated 24 h after hydration began, there were no traces of the sulfated form and the amount of released ascorbate was close to the amount present in sulfated form in cysts. The first liberated ascorbate was found in *Artemia* embryos at 16 h of hydration (Fig. 1).

The results of the two assays developed for ASS-ase activity did not agree (Table 1). Analysis of crude extracts for ASS-ase activity posed several problems (Dabrowski et al., in prep.) and assay conditions need to be ameliorated for specificity (substrate concentration, temperature, etc.) of invertebrate enzyme. However, the tendency for ASS-ase activity to increase to its maximum values at the time of hatching corraborated the results of the liberation of ascorbate from the sulfated form (Fig. 1).

AS concentration varies considerably among different *Artemia* strains (Table 2). This variation seems to be unrelated to type of reproduction, whereas differences among batches of cysts from different years are considerable. San Francisco Bay batches purchased from San Francisco Bay Brand in 1987 or that supplied in 1988 as decapsulated cysts by the Artemia Reference Center had AS concentration ranging from 162.4 to 465 µg AA·g^{-1} (Fig. 1; Table 2). The amount of liberated AA in nauplii reflects the amount of AS present in cysts, less a correction for shell weight in cysts.

Decapsulation of *Artemia* cysts which involves partial hydration for 2 h at 25°C, treatment in decapsulation solution (NaOCl), and washing (Sorgeloos et al. 1983) did not lead to the liberation of AA from the sulfated form (Table 3). Removal of the shell increased concentration of AS in decapsulated cysts compared with dry cysts. Drying of decapsulated cysts to achieve water level below 10% in dark seems to protect degradation of ascorbate in comparison with other treatments.

Discussion

The data presented in Fig. 1 show that the onset of AS breakdown occurs at 5–6 h after hydration begins. Clegg (1976) demonstrated that the onset of respiration by hydrated *Artemia* embryos takes place at 33–36% moisture content. Cysts incubated in 0.5 M sodium chloride achieved the required hydration level after 30 min of incubation. The onset of respiration in cysts undergoing hydration initiates the metabolism and syn-

TABLE 1. ASS-ase activity in *Artemia* embryos (mean ± SD, n = 4, expressed as µg AA·h^{-1}·g wet weight^{-1}). Embryos were incubated at 25 ppt, 28°C, and mass hatching took place at 13 h. n.d. = not detectable.

	Hydration time (h)							
	3	5	7	9	11	13	15	24
Dry mater (%)	29.6 ± 1.1	28.8 ± 0.2	33.2 ± 0.5	29.0 ± 0.6	29.0 ± 0.6	23.7 ± 4.1	25.1 ± 3.1	16.5 ± 2.8
Direct assay	n.d.	n.d.	n.d.	n.d.	6.0 ± 1.1	6.33 ± 1.6	10.1 ± 2.8	n.d.
DCIP assay	264 ± 20	323 ± 37	547 ± 28	888 ± 18	739 ± 58	709 ± 12	752 ± 51	523 ± 24

TABLE 2. AS or AA concentration in batches of *Artemia* cysts and nauplii from different populations (mean of three determinations, expressed as μg AA·g dry matter^{-1}).

Source of cysts	Type of *Artemia* strain B: bisexual P: Parthenogenetic	Cysts[a]	Nauplii (24 h)[b]
Shark Bay, Australia	P	164.8	n.a.
Chaplin Lake, Canada	B	189.1	n.a.
Lavalduc, France	P	242.6	n.a.
Great Salt Lake, Utah, USA	B	261.6	449.0 83.0
San Francisco Bay, California, USA	B	162.4	219.8 34.9
People's Republic of China, Tientsin	P	244.4	n.a.

[a]AS expressed as AA.
[b]Total ascorbate and DHA (see Materials and Methods) (n.d. = not analyzed).

TABLE 3. Effect of decapsulation at various conditions on *Artemia* cysts ascorbate status (means of two determinations).

Method	AS (μg AA·g^{-1})
Dry, nondecapsulated cysts	428.5
Decapsulated, in brine	285.3
Decapsulated, dried in light	558.3
Decapsulated, dried in dark	578.5
Decapsulated, dried at 60°C	551.0
Decapsulated, inactivated by UV irradiance	554.5

thesis of macromolecules. Although the data representing dry matter content during *Artemia* cyst incubation (Table 1) do not reflect physiological hydration, it seems that no change of water content between 3 and 13 h indicates that no more hydration takes place before the shell is removed. Considerable increase of ASS-ase activity between 3 and 9 h of incubation corroborates the increase of liberated ascorbate in *Artemia* embryos (Fig. 1).

Arylsulfatases which show activity against AS are secreted to the extracellular spaces in the digestive gland of molluscs (*Haliotis iris*) (Clark and Jowett 1988), and they may play a significant role in proteoglycan metabolism and consequently in cell locomotion during morphogenesis of sea urchin (*Hemicentrotus pulcherrimus*) embryos (Sasaki et al. 1987). *Artemia* arylsulfatase seems to play a very specific role in mobilization of AA during embryonic development. However, activity of 1.32–3.7 μg AA·mg·protein^{-1}·h^{-1} does not suggest higher activity than that found in fish liver (Dabrowski et al. 1990b). Arylsulfatases in invertebrates resemble the vertebrate arylsulfatases that are inhibited by phosphate and sulfate ions (Sasaki et al. 1987; Davolio et al. 1986; Carlson et al. 1977). This has been avoided in preparation of *Artemia* crude extracts. However, macromolecules such as phospholipids and some nucleotides have been shown to inhibit arylsulfatase in invertebrates (Davolio et al. 1986). I believe that powerful, high molecular weight inhibitors acted in the present assay, and work must be done to eliminate these factors and measure ASS-ase activity in *Artemia* during undisturbed embryonic development.

Variation in ascorbate concentration during embryogenesis of other crustaceans has been interpreted as the ability to synthesize ascorbate (Guary and Guary 1975). However, stable forms of ascorbate were not analyzed and in fact we do not know how common is the phenomenon of AS in resting eggs of invertebrates and eggs which do not undergo diapause. Another interesting feature of *Artemia* embryonic development is aerobic dormancy which may be provoked by environmental anoxia (Hand and Gnaiger 1988). During cessation of metabolism, energy flow is suppressed to 2.4% of aerobic values for several hours. The shutdown of energy metabolism does not seem to arrest all enzymatic reactions, however, at the time of arrest (9–17.5 h at 25°C), ascorbate mobilization might proceed at a somewhat slower rate securing sufficient ascorbate reserves at hatching.

Feeding habits of crustacean zooplankton are reflected in the ascorbate concentration in the body, and starvation of marine copepods leads to ascorbate depletion of their body (Poulet et al. 1989; Hapette and Poulet 1990). Since *Artemia* shows high genetic and life history variability (Browne 1988), the variation in AS concentration in cysts may simply reflect adult nutrition during egg production. The variation in egg size and number, the timing and spacing of clutches, and sexual or asexual reproduction is reflected in chemical characteristics of cysts and nauplii (Leger et al. 1986). Ascorbate metabolism is no exception to this variation (Table 2) and changes between populations (strains) and broods from different years might significantly influence AS concentrations in cysts, ascorbate concentration in nauplii, and consequently nutritional value to larval fish.

Decapsulated cysts of *Artemia* gave very high and consistent survival when offered to larval common carp and African catfish during the initial 2 wk of feeding (Verreth et al. 1987; Vanhaecke et al. 1990). The analysis of decapsulated cysts (Table 3) revealed that there is no liberated AA present in the embryos. Given that AS present in *Artemia* cysts and synthesized AS are identical compounds and the inability of fish, particularly larval and juveniles, to utilize AS as source of vitamin C (Dabrowski et al. 1990a, 1990b), feeding decapsulated cysts to larval fish might lead to vitamin C deficiency. Further studies are required to compare ascorbate status in larval and juvenile fish offered live nauplii and decapsulated cysts.

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References

BAKER, E. M., D. C. HAMMER, S. C. MARCH, B. M. TOLBERT, AND J. E. CANHAM. 1971. Ascorbic sulfate: a urinary metabolite of ascorbic acid in man. Science (Wash., DC) 173: 826–827.

BOND, A. D., B. W. MCCLELLAND, J. R. EINSTEIN, AND F. J. FINAMORE. 1972. Ascorbic acid-2-sulfate of the brine shrimp, *Artemia salina*. Arch. Biochem. Biophys. 153: 207–214.

BROWNE, R. A. 1988. Ecological and genetic divergence of sexual and asexual brine shrimp (*Artemia*) from the Mediterranean Basin. Natl. Geogr. Res. 4: 548–554.

CARLSON, R. W., M. DOWNING, AND B. M. TOLBERT. 1977. Purification and kinetic and physical characterization of bovine ascorbate-2-sulfate sulhydrolase. Biochemistry 16: 5221–5227.

CLARK, A. G., AND D. A. JOWETT. 1988. Purification and properties of an arylsulphatase from the digestive gland of *Haliotis iris*. Comp. Biochem. Physiol. 89B: 409–417.

CLEGG, J. S. 1976. Interrelationship between water and metabolism in *Artemia cysts* — III. Respiration. Comp. Biochem. Physiol. 53A: 89–93.

DABROWSKI, K., AND S. HINTERLEITNER. 1989. Applications of a simultaneous assay of ascorbic acid, dehydroascorbic acid and ascorbic sulfate in biological materials. Analyst 114: 83–87.

DABROWSKI, K., N. EL-FIKY, G. KOCK, M. FRIGG, AND W. WIESER. 1990a. Requirement and utilization of ascorbic acid and ascorbic sulfate in juvenile rainbow trout. Aquaculture 91: 317–337.

DABROWSKI, K., R. LACKNER, AND C. DOBLANDER. 1990b. Effect of dietary ascorbate on the concentration of tissue ascorbic acid, dehydroascorbic acid, ascorbic sulfate, and activity of ascorbic sulfate sulfohydrolase in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 47: 1518–1525.

DAVOLIO, E., L. LANDINI, N. VOLPI, M. G. DUBOIS, M. MASSON, A. PEDERZOLI, AND L. BOLOGRANI. 1986. Regulation of arylsulphatases: inhibition of arylsulphatase from *Haliotis rufusensis* by phosphoric esters and shift of optimal pH depending on temperature. Comp. Biochem. Physiol. 84B: 261–267.

DESJARDIN, L. M., J. D. CASTELL, AND J. C. KEAN. 1985. Synthesis of dehydroascorbic acid by subadult lobster (*Homarus americanus*). Can. J. Fish. Aquat. Sci. 42: 370–373.

GOLUB, A. L., AND F. J. FINAMORE. 1972. Ascorbic acid sulfate metabolism in the brine shrimp. Fed. Proc. 31: 2765.

GUARY, M. M., AND J. C. GUARY. 1975. Variations de la teneur en acide ascorbique dans les oeufs de *Cancer pagrus* et *Palaemon serratus* (Crustacea: Decapoda) au cours de l'embryogenese. Mar. Biol. 32: 357–363.

HAND, S. C., AND E. GNAIGER. 1988. Amerobic dormancy quantified in *Artemia* embryos: a calorimetric test of the control mechanism. Science (Wash., DC) 239: 1425–1427.

HAPETTE, A. M., AND S. A. POULET. 1990. Variation of vitamin C in some common species of marine plankton. Mar. Ecol. Prog. Ser. 64: 69–79.

HORNIG, D. 1975. Distribution of ascorbics acid metabolites and analogues in man and animals. Ann. N.Y. Acad. Sci. 258: 103–117.

LEGER, PH., D. A. BENGTSON, P. SORGELOOS, K. L. SIMPSON, AND A. D.BECK. 1986. The nutritional value of *Artemia*: are view. Oceanogr. Mar. Biol. Annu. Rev. 24: 521–623.

MEAD, C. G., AND F. J. FINAMORE. 1969. The occurrence of ascorbic acid sulfate in brine shrimp. Biochemistry 8: 2652–2655.

MILLER, D. AND A. G. MCLENNAN. 1988. The heat shock response of the cryptobiotic brine shrimp *Artemia* — I. A comparison of the thermotolerance of cysts and larvae. J. Therm. Biol. 13: 119–123.

MUMMA, R. O., AND A. J. VELANGIERI. 1972. Isolation of ascorbic acid 2-sulfate from selected rat organs. Biochim. Biophys. Acta 273: 249–253.

POULET, S. A., A. M. HAPETTE, R. B. COLE, AND J. C. TABET. 1989. Vitamin C in marine copepods. Limnol. Oceanogr. 34: 1325–1331.

SASAKI, H., K. AKASAKA, H. SHIMADA, AND T. SHIROYA. 1987. Purification and characterization of arylsulfatase from sea urchin (*Hemicentrotus pulcherrimus*) embryos. Comp. Biochem. Physiol. 88B: 147–152.

SORGELOOS, P., E. BOSSUYT, P. LAVENS, PH. LEGER, P. VANHAECKE, AND D. VERSICHELE. 1983. The use of brine shrimp *Artemia* in crustaceans hatcheries and nurseries, p. 71–96. *In* J. P. McVey [ed.] Crustacean aquaculture. Vol. 1. CRC Press, Inc., Boca Raton, FL.

VANHAECKE, P., L. DEVRIEZE, W. TACKER, AND P. SOREGLOOS. 1990. The use of decapsulated cysts of brine shrimp *Artemia* as direct food for carp (*Cyprinus carpio*) larvae. J. World Aquacult. Soc. 21: 257–262.

VERRETH, J., V. STORCH, AND H. SEGNER. 1987. A comparative study on the nutritional quality of decapsulated *Artemia* cysts, micro-encapsulated egg diets and enriched dry feeds for *Clarias goriepinus* (Burchell) larvae. Aquaculture 63: 269–282.

Use of the Morphoedaphic Index to Predict Nutrient Status and Algal Biomass in Some Canadian Lakes

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Chow-Fraser, P. 1991. Use of the morphoedaphic index to predict nutrient status and algal biomass in some Canadian lakes. Can. J. Fish. Aquat. Sci. 48:1909–1918.

The underlying assumptions of the morphoedaphic index (MEI; total dissolved solids (TDS)/mean depth), which has been used to predict fish yields in lakes, and its power for predicting biomass at lower trophic levels were examined using published data. The assumptions included the relationship between mean depth of lakes and various hydrologic characteristics (flushing rate and stratification regime), water transparency characteristics (water color and turbidity), and the stoichiometric relationship among ions (expressed as a proportion between TDS and the concentration of primary nutrients, total phosphorus (TP) and total nitrogen (TN)). Although these basic assumptions could be supported empirically, the predictive power of the MEI became progressively weakened with increasing trophic level. MEI accounted for up to 85% of the variation in TP and TN, less than 50% of the variation in [Chl a], and none of the variation in the biomass of herbivorous zooplankton. The functions relating TDS to both TP and TN were fundamentally different: as lakes increased in salinity, the TN:TP ratio decreased dramatically so that TP almost exceeded TN concentrations in extremely saline lakes. This necessitated the development of separate MEI–nutrient relationships for saline (TDS > 1000 mg/L) and nonsaline lakes.

Au moyen de résultats qui ont paru, nous avons examiné les hypothèses qui sous-tendent l'utilisation de l'indice morphoédaphique (IME; matières totales dissoutes/profondeur moyenne), indice qui a servi à prévoir le rendement des lacs en poisson; nous avons examiné aussi l'efficacité de cet indice comme moyen de prévision de la biomasse à des niveaux trophiques inférieurs. Les hypothèses considérées portent notamment sur les rapports entre la profondeur moyenne des lacs et différentes caractéristiques hydrologiques (vitesse de renouvellement de l'eau et régime de stratification), la transparence de l'eau (couleur de l'eau et turbidité) ainsi que les proportions stochiométriques entre les différents ions (exprimées en rapport entre les matières totales dissoutes et la concentration des éléments nutritifs primaires, du phosphore total (PT) et de l'azote total (NT)). Quoique ces hypothèses fondamentales trouvaient une justification empirique, il demeure que la capacité de prévision de l'IME s'affaiblit progressivement à mesure qu'on progresse dans l'échelle trophique. L'IME permettait de justifier jusqu'à 85 % des variations de PT et de NT, il permettait de justifier moins de 50 % des variations de la concentration en chlorophylle *a*, et il ne permettait de justifier aucunement les variations de la biomasse du zooplancton herbivore. Les fonctions qui établissaient une relation entre les matières totales dissoutes et le PT ainsi que le NT étaient fondamentalement différentes : à mesure qu'on progressait dans l'échelle de salinité des lacs, le rapport NT:PT diminuait de manière spectaculaire, au point que la concentration en PT dépassait presque la concentration en NT dans les lacs extrêmement salés. Il a donc fallu développer des fonctions distinctes pour mettre en relation l'IME et les éléments nutritifs dans les lacs salés (matières totales dissoutes supérieures à 1 000 mg/L) qui sont distinctes de celles des lacs non salés.

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The morphoedaphic index (MEI; Ryder 1965), calculated as the concentration of total dissolved solids ([TDS]) divided by mean depth, is commonly used by fisheries biologists to predict potential fish yield in lakes (Ryder et al. 1974; Matuszek 1978; Kerr 1982). Although the original MEI was derived empirically (Ryder et al. 1974; Ryder 1982), the conceptual framework that underlies this model include the following explicit assumptions: (1) bedrock geology and climate largely determine the [TDS] entering lakes, and TDS is a surrogate for essential nutrients such as phosphorus and nitrogen which control the lake's primary productivity; and (2) mean depth, an overall parameter of lake morphometry, affects the distribution of organisms and is a surrogate for hydrological characteristics such as stratification (temperature regime), nutrient circulation, and dilution, all of which affect how energy is processed within the water column (i.e. mean depth is representative of a nutrient and energy sink in lakes; Rawson 1951, 1952; Northcote and Larkin 1956; Ryder 1965; Hanson and Leggett 1982).

In the two and a half decades since its introduction, the MEI has been used widely in fisheries management through appropriate modifications accounting for regional and global climatic differences (Henderson et al. 1973; Ryder et al. 1974; Schlesinger and Regier 1982) and modifications accounting for differences in hydraulic retention time and ionic composition of lake water (Jenkins 1982). Recently, its usefulness in aquatic sciences has been under scrutiny. On technical grounds, criticisms include the inaccurate prediction limits around estimates due to artificial correlation between dependent and independent variables in the model (Schneider and Haedrich 1989) and the biased nature of ratios due to spurious correlations and the use of inappropriate null models (Jackson et al. 1990). Other crit-

TABLE 1. Range and mean values (± SE) corresponding to studies assembled for this investigation. Mean values are in parentheses. TP = [total phosphorus] (μg/L); TN = [total nitrogen] (μg/L); CHL = [Chl *a*] (μ/L); n/a = not available.

Location	Investigators and study	No. of lakes	TP	TN	CHL	Mean depth (m)
British Columbia coast	Shortreed and Stockner 1981; Stockner et al. 1980	21	3.0–18.0 (5.2 ± 3.1)	118–208 (158 ± 28.5)	0.8–2.0 (1.3 ± 0.5)	13–212 (55.2 ± 51.8)
	Stockner and Shortreed 1985	4	1.0–4.0 (1.9 ± 1.4)	120–126 n/a	0.7–3.3 (1.5 ± 1.2)	39–59 (46.0 ± 15.8)
Central Alberta	Chow-Fraser and Trew 1990	16	14.0–66.0 (30.9 ± 16.4)	546–1666 (1077 ± 300.5)	2.6–25.0 (10.2 ± 7.5)	2.9–53.0 (11.5 ± 12.4)
	Prepas and Trew 1983[a]	17	11.0–152.0 (46.8 ± 34.7)	495–4104 (1669 ± 1088)	1.7–80.6 (23.7 ± 21.4)	3.2–19.4 (8.2 ± 5.0)
	Bierhuizen and Prepas 1985	20	21–14077 (2215 ± 4158)	1094–10736 (4781 ± 2274)	3.5–90.0 (26.2 ± 30.4)	0.9–8.0 (3.6 ± 2.1)
Nova Scotia	Kerekes 1975	20	2.8–10.9 (7.4 ± 2.9)	n/a	0.3–2.9 (1.7 ± 0.7)	1.4–74.0 (6.6 ± 16.0)
Quebec	Pace 1984	12	3.4–55.7 (13.0 ± 13.9)	n/a	1.2–28.6 (5.3 ± 7.5)	2.9–44.0 (17.8 ± 14.3)
	Chow-Fraser and Maly 1991	8	13.4–47.2 (28.7 ± 12.4)	n/a	2.3–8.95 (4.8 ± 2.5)	n/a
	Bird and Kalff 1984[b]	2	10.0	n/a	4.0–4.6	n/a
	Chow-Fraser and Duthie 1987	4	6.7–19.6	n/a	1.3–2.1	n/a
Ontario	Zimmerman et al. 1983	37	3.0–18.0 (7.7 ± 3.9)	203–673 (350 ± 94)	0.3–7.5 (2.2 ± 1.5)	0.9–20.1 (7.6 ± 4.3)

[a]Excludes lakes included in Chow-Fraser and Trew (1990) and Bierhuizen and Prepas (1985).
[b]Only one value of TP available.

TABLE 2. Summary of Pearson correlation coefficients (see Methods for explanation of abbreviations and units). Only significant correlation coefficients are reported. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. All values were log-transformed except pH.

	Mean depth	Max depth	*A*	FR	TURB	COLOR	TDS	COND
Max depth	+0.91****							
A	+0.45****							
FR	−0.49****	−0.53****	−0.37**					
TURB			+0.63****					
COLOR	−0.59****				+0.69***			
TDS	−0.79****			−0.63****	−0.32**	+0.46**		
COND	−0.43****	−0.25*			−0.36*		+0.99****	
pH	−0.46****	−0.26*		−0.50**	−0.48**		+0.90****	+0.90****

icisms concern the ineffectiveness of the MEI to predict fish production when compared with other indices based on surface area (Youngs and Heimbuch 1982) or lake productivity indicators (Hanson and Leggett 1982; Prepas 1983; Downing et al. 1990).

Since one of the basic assumptions of the MEI is the presumed proportionality between TDS and primary nutrients (based on the stoichiometric relationship among ions in lake water), it is surprising that in some lakes, nutrient concentration could accurately predict yield/production when the MEI could not. To understand how the MEI fails to predict fish yield in these situations, there must be a better understanding of the general relationship between MEI and the concentrations of primary nutrients (i.e. total phosphorus (TP) and total nitrogen (TN)). It is also important to investigate the effectiveness of the MEI for predicting biomass at lower trophic levels, since primary productivity was a good predictor of fish yield in some lakes (Downing et al. 1990).

In this paper, I have assembled data from the literature to first examine the two basic assumptions of the MEI: the stoichiometric relationship between TDS and primary nutrients and the relationship between mean depth and variables that may affect lake productivity such as hydrologic characteristics and water transparency. I have purposely included lakes that cover a large geographical area to test the general effectiveness of the MEI for predicting nutrient concentration in a wide range of lake types. Although all study lakes occur in Canada, I have included lakes from the United States in an independent lake set to test the general applicability of relationships that emerge from this study. Secondly, I examine the relationship between MEI and plankton biomass to evaluate the general applicability of the MEI for predicting energy flow at lower trophic levels. By testing these underlying assumptions, I hope to provide a better understanding of the nature and source of disparities between the MEI and biomass at higher trophic levels.

Methods

I have focused on Canadian lakes in this paper primarily to minimize the effect of large-scale climatic variations on the

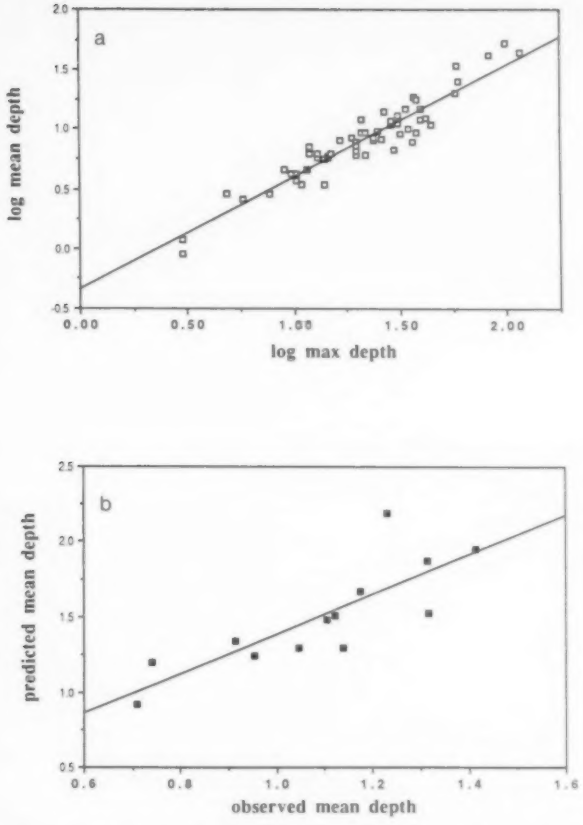


FIG. 1. (a) Log mean depth (m) versus log maximum depth (m) for study lakes. Equation for least-squares regression line is log mean depth = 0.940 (± 0.058) log max depth −0.349 (r^2 = 0.82; n = 60; P = 0.0001). (b) Predicted versus observed mean depth (m) from Rawson's (1960) study. The line indicates that predicted = observed.

MEI (Schlesinger and Regier 1982); however, I have relaxed all restrictions pertaining to basin size, lake depth and elevation, salinity, turbidity, and flushing rate. The 161 lakes included in the study are located throughout Canada from Nova Scotia to British Columbia (Table 1). Not all of these lakes were included in every analysis because some variables were not measured or were not made available. Compared with other published studies in which the MEI has been evaluated (e.g. Ryder 1965; Matuszek 1978; Jones and Hoyer 1982; Prepas 1983), this study contains a wide range of values.

Variables Included

As mean depth (metres) is only one of several morphometric parameters, I included maximum depth (max depth; metres) and lake surface area (A; square kilometres) in my analysis. I chose flushing rate (FR; per year) and the lake's stratification characteristic (STRAT) as the main hydrologic parameters because they are readily reported and both play a role in dilution and regeneration of nutrients in the water column (Murphy 1962; Kerekes 1973; Nurnberg and Peters 1984). As a measure of reduced water transparency resulting from nonalgal sources, I chose turbidity (TURB; JTU) and color (Pt units), which are

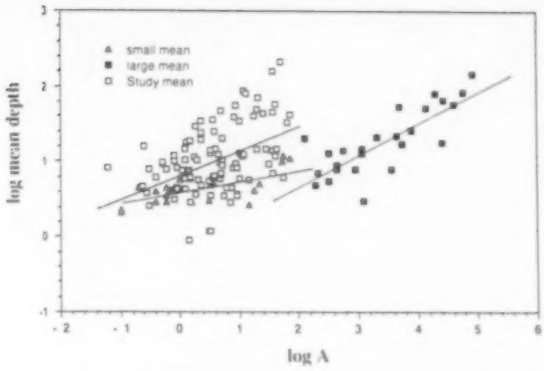


FIG. 2. Log mean depth (m) versus log lake surface area (A; km^2). Open symbols are data for small lakes ($A \leq 100$ km^2) and closed symbols are those for large lakes ($A > 100$ km^2). Open squares are data from this study; closed squares and open triangles are those from the independent dataset (see Methods). Equation for regression line through open squares is log mean depth = 0.312 (± 0.058) log A + 0.768 (r^2 = 0.20; n = 119; P = 0.0001). Equation for regression line through open triangles is log mean depth = 0.161 (± 0.033) log A + 0.597 (r^2 = 0.50; n = 26; P = 0.0001). Equation for regression line through closed squares is log mean depth = 0.413 (± 0.064) log A −0.135 (r^2 = 0.65; n = 25; P = 0.0001).

TABLE 3. Mean depth (m) associated with stratification characteristics of study lakes. All categories are significantly different from each other as indicated by an ANOVA and multiple range test ($P < 0.05$).

Mixing regime	n	Mean depth	SE
Nonstratified	53	3.06	1.14
Weakly stratified	28	10.81	1.18
Stratified	47	19.36	1.10

both implicated in suppressing primary productivity (Murphy 1962; Janus and Vollenweider 1981; Hoyer and Jones 1983).

Because TDS (milligrams per litre) was not always available, I also used specific conductance (COND; microsiemens per centimetre)) in the numerator of the MEI because the latter is widely used as a measure of mineral salt concentration and is well correlated with TDS (Bayly and Williams 1973; Foster et al. 1981). pH was also included in my analysis because it can be used as a surrogate of TDS and COND when neither is available (r = + 0.90 for both variables; Table 2). The two primary nutrients included were TP (micrograms per litre) and TN (micrograms per litre) whereas biotic variables included [Chl a] (CHL; micrograms per litre) and the biomass of phytoplankton (PHYTO; micrograms per litre) and herbivorous zooplankton (HERB; micrograms per litre). Lakes designated as saline in this study are those of Bierhuizen and Prepas (1985) who described these lakes as having high TDS (values >1000 mg/L) with high salt concentration rather than humic substances.

Independent Data Set

To test the generality of empirical relationships obtained in this study, I assembled values from previously published studies on morphoedaphic factors and fish yield to form an independent data set. The data set included TP, CHL, COND, mean depth, and A from Jones and Hoyer (1982), mean depth and A

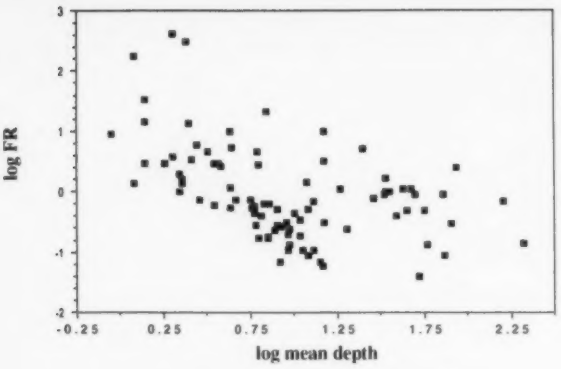


FIG. 3. Log flushing rate (FR) versus log mean depth (m).

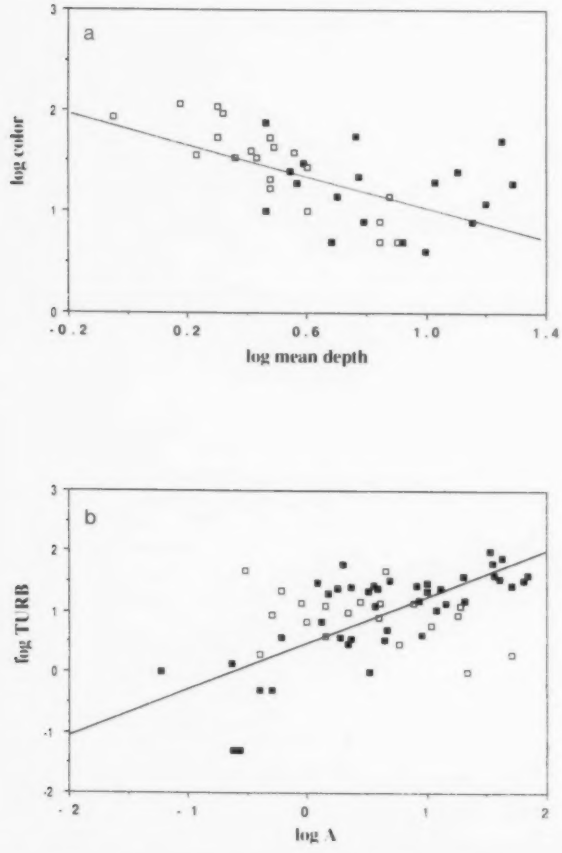


FIG. 4. (a) Log color versus log mean depth (m). Open symbols correspond to saline lakes and closed symbols correspond to nonsaline lakes (TDS > and ≤ 1000 mg/L, respectively). Equation for regression through both open and closed symbols is log color = −0.778 (± 0.174) log mean depth + 1.845 (r^2 = 0.35; n = 39; P = 0.0001). (b) Log turbidity (TURB; JTU) versus log surface area (A; km^2). Key for symbols are as in Fig. 4a. Equation for regression through both open and closed symbols is log TURB = 0.607 (± 0.096) log A + 0.600 (r^2 = 0.40; n = 62; P = 0.0001).

from Youngs and Heimbuch (1982), TDS from Ryder (1965) and Matuszek (1978), and mean depth, max depth, STRAT, and the biomass of netplankton (kilograms dry weight per hectare) from Rawson (1960).

Statistical Treatment

All of the data except pH and STRAT were first $\log_{10}$-transformed (referred to as log in the remainder of the text) to stabilize the variance and normalize the residuals. Linear and multiple regression analyses were performed and, where appropriate, subsequent analysis of covariance was carried out (Sokal and Rohlf 1981). In all cases, the criterion level for significance was 0.05.

Results and Discussion

Testing the Assumptions of the MEI

Although mean depth is the variable Ryder (1965) used in his original formulation of the MEI, other morphometric variables may also be used. Youngs and Heimbuch (1982), for example, suggested that mean depth was only a surrogate of lake surface area (A), and that A should instead be used. This conclusion was recently reinforced by studies on Finnish lakes (Ranta and Lindstrom 1989, 1990). The deepest spot in the lake (max depth) may also be used as a surrogate if the relationship between mean and maximum depth is known. I found a highly significant correlation between mean depth and max depth (Fig. 1a). The good predictive power of this regression model was demonstrated when I tested it using independent data from Rawson's (1960) study (Fig. 1b). The good fit of values to the line of unity indicates that max depth may in general be a good substitute for mean depth and that an alternative MEI may be calculated with either max depth or values estimated from max depth.

By comparison, the relationship between mean depth and A was more complex. The regression in this case only explained 20% of the overall variability in mean depth, and the equation only seemed to apply to small lakes (≤100 km^2; open symbols in Fig. 2). This was obvious when I plotted values from the independent data set (see Methods; closed squares and open triangles) together with those in the study (open squares). The study data overlapped completely with those of small lakes (open triangles) but did not at all overlap with those of large lakes (closed squares). Slopes of the regression lines through study data and that through large lakes in the independent data set were not significantly different ($P > 0.05$); an analysis of covariance further indicated that their intercepts were significantly different ($P < 0.05$). Because A was only correlated with mean depth for lakes within respective size categories, it is doubtful that Youngs and Heimbuch's (1982) proposal to use A as a surrogate of mean depth can be generally applied without some qualifications.

Morphometry versus hydrology

There was an apparent relationship between mixing regime of lakes and their mean depth (Table 3). On average, nonstratified lakes were shallow (1–8 m) whereas strongly stratified lakes were deep (4–200 m), while weakly stratified lakes were intermediate in depth (5–158 m). Average mean depths of nonstratified systems was only 3.1 m, compared with 19.4 m for stratified lakes. Therefore, mean depth appears to be a rough indicator of the mixing regime in lakes.

TABLE 4. Summary of significant regression equations between TP and TN and some other variables. SE = standard error of the regression coefficient.

Y variable	*X* variable	*n*	r^2	*P*	*b*	SE	*a*
log TP	log max depth	65	0.29	0.0001	−0.491	0.097	1.732
log TP	log mean depth	129	0.27	0.0001	−0.812	0.120	1.965
log TN		97	0.39	0.0001	−0.712	0.092	3.499
log TP	log COLOR	48	0.17	0.0042	0.794	0.265	0.851
log TN		36	0.46	0.0001	0.583	0.109	2.653
log TP	log TDS	77	0.81	0.0001	1.012	0.056	−0.875
log TN		63	0.76	0.0001	0.603	0.043	1.556
log TP	log COND	78	0.68	0.0001	0.850	0.066	−0.509
log TN		71	0.83	0.0001	0.545	0.030	1.607
log TP	pH	102	0.50	0.0001	0.455	0.045	−2.067

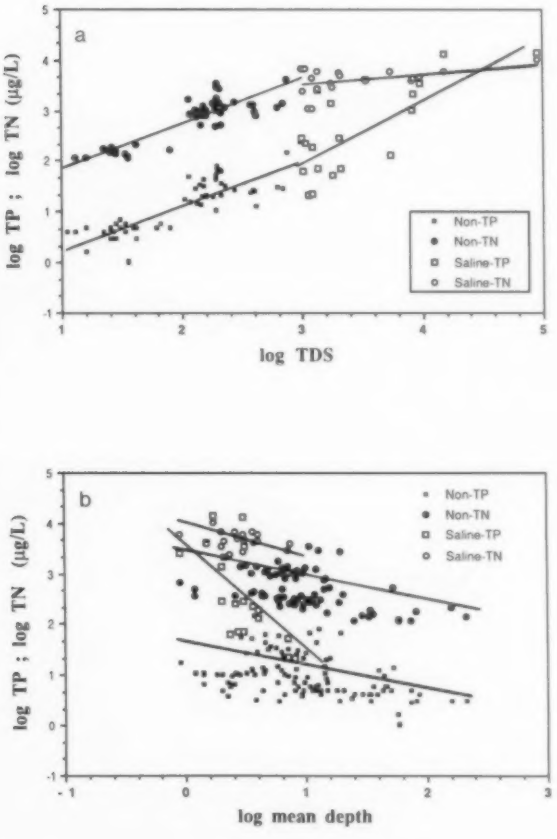


FIG. 5. Nutrient concentration versus (a) TDS for both saline and nonsaline lakes and (b) log mean depth (m). (a) Equations for nonsaline lakes are log TN = 0.862 (± 0.077) log TDS + 1.025 (r^2 = 0.75; n = 43; P = 0.0001) and log TP = 0.905 (± 0.081) log TDS − 0.669 (r^2 = 0.69; n = 57; P = 0.0001). Equations for saline lakes are log TN = 0.210 (± 0.103) log TDS + 2.899 (r^2 = 0.19; n = 20; P = 0.05) and log TP = 1.221 (± 0.271) log TDS − 1.584 (r^2 = 0.53; n = 20; P = 0.0003). (b) Equations for nonsaline lakes are log TN = −0.402 (± 0.087) log z_s + 3.073 (r^2 = 0.22; n = 77; P = 0.0001) and log TP = −0.368 (± 0.074) log z_s + 1.34 (r^2 = 0.19; n = 109; P = 0.0001). Equations for saline lakes are log TN = −0.584 (± 0.195) log z_s + 3.903 (r^2 = 0.33; n = 20; P = 0.008) and log TP = −2.067 (± 0.671) log z_s + 3.615 (r^2 = 0.35; n = 20; P = 0.006).

FR varied inversely with all three morphometric variables, mean depth, max depth, and *A* (Table 2). Shallow, small lakes appear to have a higher FR than deep, large lakes (Fig. 3). This may be because drainage ratio (drainage area to lake area) is the chief determinant of FR (Rasmussen et al. 1989) and thus the weak correlation between FR and these morphometric variables is likely due to collinearities with drainage ratio. Another explanation may be that for any given precipitation regime, drainage area divided by lake volume defines FR. Therefore, the high correlation between area and volume will undoubtedly lead to a high correlation between drainage area and lake area. In general, FR may simply follow the allometric principles detailed by Schnieder and Haedrich (1989).

Morphometry versus water transparency

Rasmussen et al. (1989) found that most highly colored lakes were small, rapidly flushed, shallow headwater lakes with large, low-sloped catchment. In this study, color was also negatively correlated with mean depth (Fig. 4a); however, it was not significantly correlated with lake area, although this may only be a consequence of the smaller sample size in this study. By comparison, TURB was significantly correlated with *A* (Fig. 4b), but not with mean depth. Thus in this study, large lakes tended to be more turbid than small lakes, regardless of mean depth. Unfortunately, the relatively small sample size (n = 62) in this study precludes drawing any conclusions.

These results confirm that mean depth generally yields information regarding the mixing regime and flushing rate of lakes and thus substantiate the basic assumption that mean depth is an overall surrogate of hydrologic characteristics. In addition, mean depth also reflects water transparency, since shallow lakes tend to be more highly colored and more turbid. The significant correlation of mean depth with apparently antagonistic effects is interesting. For instance, shallow lakes may be more productive because they do not stratify and the entire water column is well lit, but this effect may be cancelled by the higher FR and more turbid water that are also associated with shallow lakes, and that may bring about reduced algal productivity. Further refinement of the relationship between mean depth and the standing stock of lake biota will probably depend on clarification of the relative importance of these factors in controlling primary productivity.

TDS/COND versus nutrients

The best predictors of TP and TN were TDS and COND, which described 81 and 83%, respectively, of the variability in nutrient concentrations (Table 4). Since the data set included some saline lakes that have extremely high TDS and COND

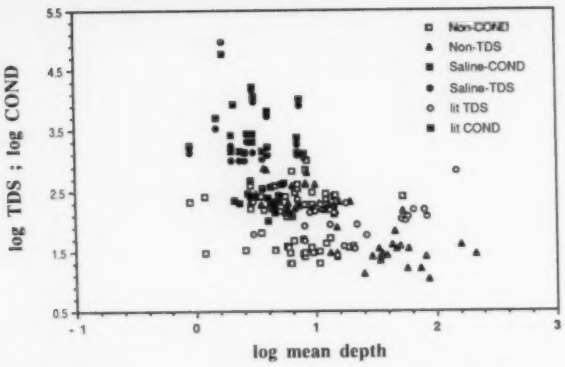


FIG. 6. Log total dissolved solids (TDS; mg/L) and log conductance (COND; μS/cm) versus log mean depth (m). Non-COND and saline-COND are conductance values for nonsaline and saline lakes, respectively; non-TDS and saline-TDS are TDS measurements for nonsaline and saline lakes, respectively; and lit-TDS and lit-COND are TDS and conductance values, respectively, for lakes in the independent data set (see Methods).

TABLE 5. Summary of significant ($\alpha = 0.05$) regression equations between TP and TN and the MEI. MEI is calculated as TDS/mean depth and MEI(C) is calculated as COND/mean depth. SE = standard error of the regression coefficient.

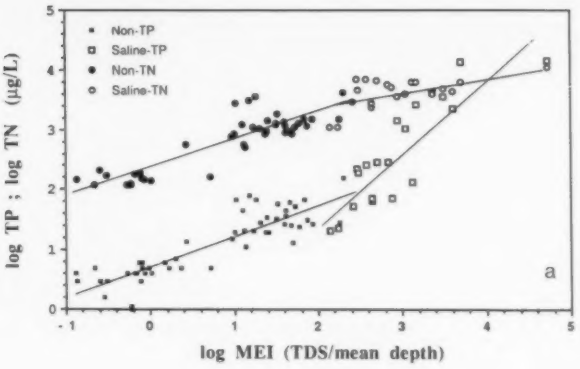
Y variable	*X* variable	*n*	r^2	*P*	*b*	SE	*a*
log TP	log MEI	73	0.83	0.0001	0.657	0.035	0.625
log TN	log MEI	62	0.84	0.0001	0.412	0.023	2.412
log TP	log MEI(C)	62	0.83	0.0001	0.821	0.049	0.069
log TN	log MEI(C)	68	0.83	0.0001	0.447	0.025	2.169

values (TDS values > 1000 mg/L; as defined by Bierhuizen and Prepas 1985), I was concerned that their inclusion biased the regression analysis. Therefore, I removed the saline lakes and performed the analysis again. Although the resulting r^2 values were reduced to 0.69 and 0.58, respectively, they were still substantially higher than those of any other variable tested.

When separate regression analyses were performed for saline and nonsaline lakes, I found that TN concentrations in saline lakes did not increase as quickly as TP in relation to TDS concentrations (Fig. 5a); in extremely saline lakes, TP even exceeded TN concentrations. Substitution with COND as the independent variable yielded the same trend. Thus, although TDS (or COND) is indeed a good surrogate for TP and TN in lakes, the function relating TDS to both nutrients are fundamentally different; as lakes increase in salinity, the TN:TP ratio decreases dramatically so that TN rather than TP becomes the limiting nutrient at high salinities (Rawson and Moore 1944).

Morphometry versus nutrients

Relationships between mean depth and nutrients were also influenced by lake salinity. TP concentrations corresponding to saline lakes declined sharply with lake depth (Fig. 5b) whereas those corresponding to nonsaline lakes showed a more gradual decline. By comparison, the slope of the regression between TN and depth did not differ significantly ($P > 0.05$) between lake types, although the intercepts were significantly higher ($P < 0.05$) for saline lakes. The steep slope of the regression line relating TP to depth for saline lakes suggests that the rate



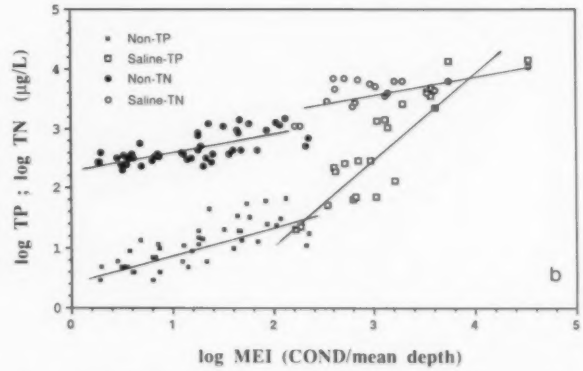


FIG. 7. Nutrient concentration versus (a) MEI (TDS/mean depth) and (b) MEI (COND/mean depth). (a) Equations for nonsaline lakes are log TN = 0.459 log MEI + 2.273 ($r^2 = 0.77$) and log TP = 0.512 log MEI + 0.716 ($r^2 = 0.77$). Equations for saline lakes are log TN = 0.253 log MEI + 2.872 ($r^2 = 0.37$) and log TP = 1.249 log MEI − 1.078 ($r^2 = 0.76$). (b) Equations for nonsaline lakes are log TN = 0.294 log MEI(C) + 2.311 ($r^2 = 0.48$) and log TP = 0.473 log MEI(C) + 0.483 ($r^2 = 0.58$). Equations for saline lakes are log TN = 0.291 log MEI(C) + 2.732 ($r^2 = 0.41$) and log TP = 1.408 log MEI(C) − 1.689 ($r^2 = 0.79$).

of enrichment in saline lakes is very rapid for lakes with shallow mean depth. This may be because accumulated TP in the sediments of saline lakes becomes more easily recirculated as lakes become more shallow. Further research should be carried out to investigate these relationships, especially since anticipated global warming may cause lake levels to drop below present levels and may trigger more problems associated with eutrophication.

Test of independence between mean depth and TDS

Since collinearity between the two parameters of the MEI would decrease the predictive power of the index, I first evaluated the relationship between TDS and mean depth before proceeding with further analyses. TDS and COND (a substitute for TDS; Table 2) were both highly correlated with mean depth (Fig. 6). On closer examination, however, it was obvious that inclusion of saline lakes biased the overall trend. When I removed these data and ran the analysis again, there was no longer a significant relationship between mean depth and the

TABLE 6. Summary of significant ($\alpha = 0.05$) regression equations between CHL, PHYTO, HERB, TP, TN, and the MEI. MEI is calculated as TDS/mean depth and MEI(C) is calculated as COND/mean depth. SE = standard error of the regression coefficient.

Y variable	*X* variable	*n*	r^2	*P*	*b*	SE	*a*
log CHL	log TP	149	0.51	0.0001	0.512	0.041	−0.087
log PHYTO		52	0.17	0.0021	1.027	0.317	1.774
log HERB		43	0.14	0.0121	0.897	0.335	1.544
log CHL	log TN	91	0.66	0.0001	0.887	0.068	−1.900
log PHYTO		47	0.59	0.0001	1.943	0.241	−1.943
log HERB		41	0.45	0.0001	2.728	0.485	−4.341
log CHL	log MEI	73	0.46	0.0001	0.311	0.040	0.339
log CHL	log MEI(C)	62	0.41	0.0001	0.336	0.052	0.097
log PHYTO		36	0.29	0.0008	0.236	0.064	2.778

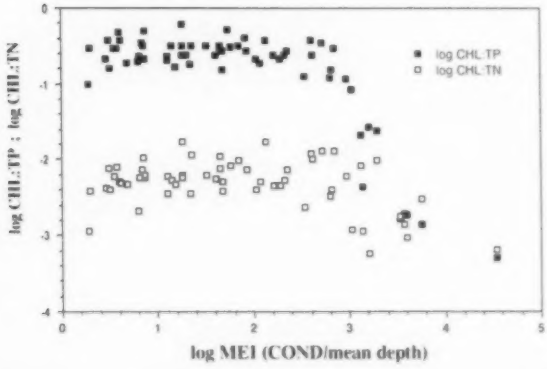


FIG. 8. Changes in CHL:TP and CHL:TN ratios with increasing MEI values.

other two variables ($P > 0.50$). This was reinforced when I superimposed literature data on the same plot and found that TDS and COND varied independently with mean depth for all non saline lakes (TDS < 1000 mg/L). This confirms the need for development of individual MEI relationships for saline and nonsaline lakes.

Relationship between MEI, Nutrients, Phytoplankton, and Zooplankton

In the previous section, I validated two assumptions of the MEI and tested the independence of the two variables in the index. I now proceed to use these variables to predict nutrient status in lakes. Since I calculated MEI using both TDS and COND in the numerator of the index, I will refer to indices calculated with TDS as MEI and those calcualted with COND as MEI(C) (Table 5). The high r^2 values indicate that both MEI and MEI(C) were good predictors of TP and TN when both saline and nonsaline lakes were included in the analysis; however, separate regressions are more ecologically meaningful for saline and nonsaline lakes because of the significant interaction between the slope of the regression and lake type (Fig. 7a and 7b). The slope for TN associated with nonsaline lakes was 0.459 whereas that for saline lakes was 0.253; by comparison, the slope for TP associated with nonsaline lakes was 0.512 whereas that for saline lakes was 1.249. Although the respective slopes could not be tested for significant differences because of nonoverlapping ranges in MEI values, it is obvious that TP concentrations increased disproportionately with MEI values > 200, while corresponding TN concentrations in this range showed almost no change in slope. Similar trends were evidenced when MEI(C) was used as the independent variable. This emphasizes the need for development of separate MEI relationships for saline lakes and supports Rawson and Moore's (1944) suggestion that TN rather than TP is the limiting nutrient for primary productivity in saline systems.

TABLE 7. Summary of significant ($\alpha = 0.05$) regression equations between CHL, TP, TN, and the MEI (MEI = TDS/mean depth; MEI(C) = COND/mean depth) for nonsaline lakes only. SE = standard error of the regression coefficient.

Y variable	*X* variable	*n*	r^2	*P*	*b*	SE	*a*
log CHL	log TP	129	0.72	0.0001	0.938	0.052	−0.509
log CHL	log TN	71	0.76	0.0001	1.184	0.079	−2.658
log CHL	log MEI	53	0.68	0.0001	0.515	0.050	0.230
log CHL	log MEI(C)	42	0.47	0.0001	0.504	0.084	−0.120

MEI versus plankton biomass

CHL is routinely used as a measure of algal standing stock in pelagic ecosystems, and although it does not reflect the species composition of the phytoplankton, it is more often available in the literature than is biomass estimate from cell counts. I have included both variables in this study, but acknowledge that analyses involving phytoplankton biomass may not be as rigorous because of the much smaller sample size. Due to limited availability of zooplankton biomass data, analyses including herbivore information are likewise restricted.

Neither the MEI nor MEI(C) was as well correlated with CHL as TN or TP (Table 6). However, TP explained more residual variation than MEI ($r^2 = 0.51$ and 0.46, respectively), while TN explained the most ($r^2 = 0.66$). This is probably because saline lakes had been included in the analysis. CHL:TP ratios were greatly reduced for saline compared with nonsaline lakes whereas CHL:TN ratios did not differ greatly between lake types (Fig. 8); consequently, CHL was better correlated with changes in TN than with changes in TP when MEI values exceeded 1000. Because inclusion of saline lakes confounded the comparison, I excluded them and performed the regression analysis of the smaller data set. (I did not run new analyses for PHYTO and HERB because there were no corresponding biomass data available for saline lakes). Analysis on the reduced data set yielded identical trends (Table 7); TP and TN were still stronger predictors, and once again, TP was only marginally better than MEI ($r^2 = 0.72$ and 0.68, respectively), while TN was the best overall predictor.

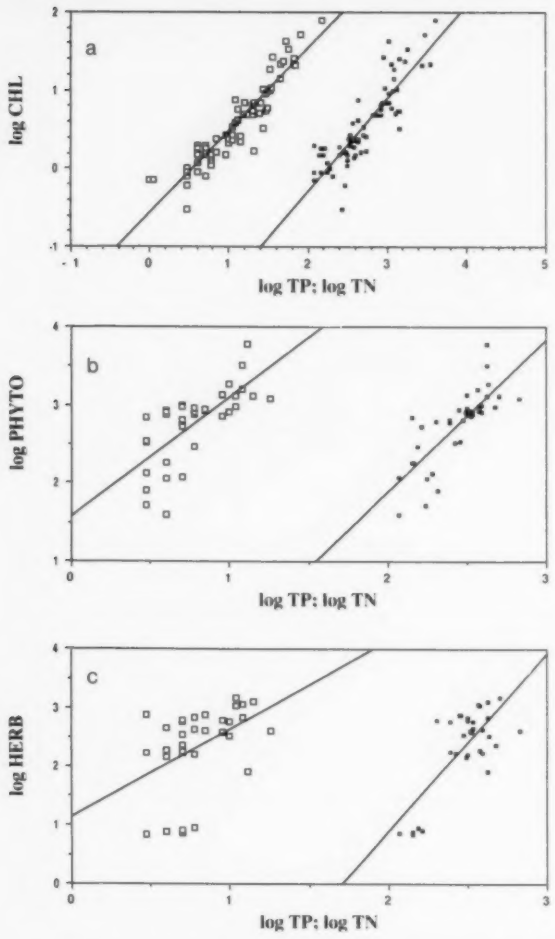


FIG. 9. (a) Log chlorophyll *a* (CHL; μg/L), (b) log phytoplankton biomass (PHYTO; μg/L), and (c) log herbivore biomass (HERB; μg/L) for nonsaline lakes in restricted lake set (see text). Equations for respective regression lines are log CHL = 1.184 (± 0.079) log TN − 2.658 (r^2 = 0.76; n = 71), log CHL = 1.051 (± 0.050) log TP − 0.567 (r^2 = 0.87; n = 71), log PHYTO = 1.943 (± 0.255) log TN − 1.994 (r^2 = 0.62; n = 38), log PHYTO = 1.518 (± 0.252) log TP − 1.585 (r^2 = 0.50; n = 38), log HERB = 3.038 (± 0.486) log TN − 5.189 (r^2 = 0.57; n = 32), and log HERB = 1.508 (± 0.545) log TP − 1.130 (r^2 = 0.20; n = 32).

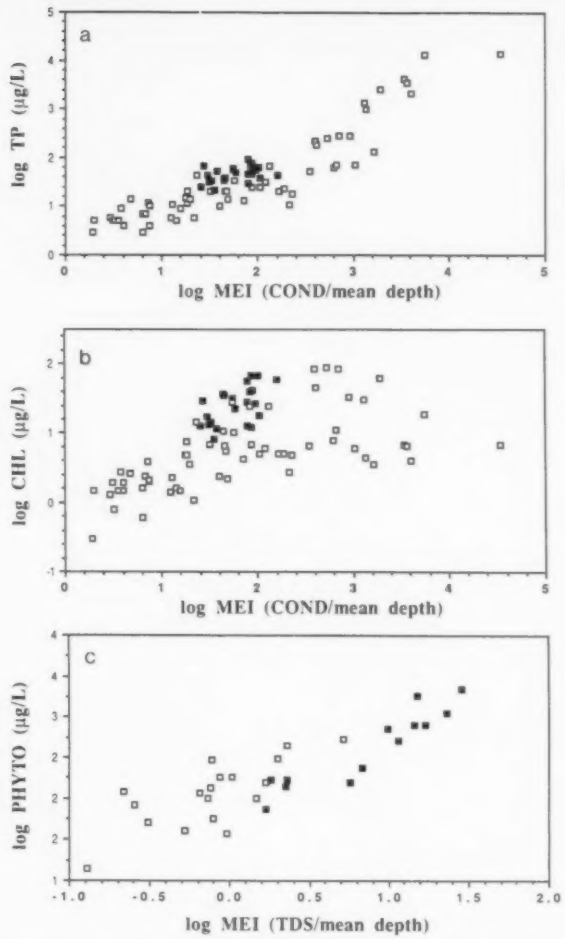


FIG. 10. (a) Log TP versus log MEI(C). Open circles are data from this study; closed circles are those from the independent data set (Jones and Hoyer 1982). (b) Log CHL versus log MEI(C). Symbols are the same as in Fig. 10a. (c) Log PHYTO versus log MEI. Open circles are data from this study; closed circles are those from the independent data set (Rawson 1960).

The predictive powers of the MEI and MEI(C) were noticeably weaker (r^2 = 0.19 and 0.29, respectively) for PHYTO but were surprisingly better than TP in this regard (r^2 = 0.17; Table 6). Consistent with previous analyses, TN emerged as the best overall predictor of algal biomass (r^2 = 0.59). There were only two significant predictors of HERB, TP and TN, with TN accounting for a larger portion of the variability (r^2 = 0.45). Neither MEI nor MEI(C) was significantly correlated with HERB. That TN emerged as the best overall predictor of CHL, PHYTO, and HERB in this study merits further attention because TP is usually considered the most limiting nutrient of algal biomass in lakes (Schindler et al. 1978). To control for possible confounding effects of different sample size, I performed regression analyses again on reduced data sets (n = 71 for CHL–nutrients, n = 38 for PHYTO–nutrients, and n = 32 for HERB–nutrients).

In the restricted data set, TP was considerably better than TN as predictor of CHL (r^2 = 0.87 versus 0.76, respectively; Fig. 9a). Thus, the superiority of TN noted in the earlier analyses probably reflected differences in sample size. By contrast, TN still emerged as the stronger predictor of PHYTO and HERB (r^2 = 0.62 versus 0.50 and 0.57 versus 0.20, respectively; Fig. 9b and 9c). Although this finding was unexpected, and the sample sizes in these latter cases were too small to allow conclusions to be drawn, more research should be conducted to determine the relative effectiveness of TP and TN for predicting standing stock at lower trophic levels when such factors as sampling time and analytical techniques can be standardized.

In this section, I have explored the relationship between MEI and nutrients and MEI and plankton biomass. Although conclusions are not possible because of limited data in some cases, it is clear that the MEI is a good predictor of TP and TN in

lakes, capable of explaining 37–85% of the variation in nutrient concentrations. However, the predictive power of the index became progressively weakened with successive trophic levels (Table 6). It was marginally weaker than TN and TP for predicting CHL, but was ineffective for predicting HERB. Since the data set was extremely small, no conclusions regarding the possible uncoupling between the MEI and primary consumers can be made. Nevertheless, the source of the uncoupling between MEI and fish yield may stem from the inability of the MEI to accurately reflect zooplankton biomass, and further studies should be conducted to address this.

Test with Independent Data Set

I used data from the independent data set to evaluate the general applicability of empirical relationships developed here for other lakes. TP and CHL from Jones and Hoyer's (1982) study of shallow productive reservoirs were plotted against MEI(C) together with data from this study (Fig. 10a and 10b). There was generally good agreement between the literature and study data. The TP values in this study bracketed those of Jones and Hoyer, although their data appeared to cluster around the central portion of the range because they were all very shallow, well-mixed productive reservoirs with little variation in mean depth (ranging from 2.1 to 5.8 m). CHL values in this independent data set also overlapped with data in the upper portion of the range in this study, and in a few instances were higher than expected based on MEI(C). Unfortunately, I was not able to confirm the effect of mixing regime on the CHL–MEI relationship; when I regressed CHL against MEI and STRAT, stratification characteristic did not account for any significant amount of the variation in CHL.

I was able to use net plankton data from Rawson's (1960) study of large deep lakes to test the general effectiveness of the MEI for predicting PHYTO. Although there was little overlap in range between Rawson's data and those in this study, the respective trends were complementary (Fig. 10c). This suggests that the relationship between MEI and PHYTO established in this study can be applied to other lake types, and investigators should be encouraged to collect contemporaneous information on nutrients, plankton biomass, and fish yield for diverse lake types to rigorously test the generality of the MEI-biota relationships.

Summary

This study confirmed that important hydrologic (i.e. mixing regime and FR) and water transparency characteristics are significantly correlated with mean depth and that mean depth is better than *A* as an overall indicator of lake morphology. It also validated the basic assumption that TDS is a good surrogate of primary nutrients in lakes. Therefore, these assumptions of the MEI can be supported empirically. Despite this, the predictive power of the MEI became progressively weakened with increasing trophic level. While it accounted for up to 85% of the variation in TP and TN, it accounted for less than 50% of the variation in CHL, and none of the variation in HERB. This is problematic because the relationships between TP and various trophic levels are well documented (e.g. TP versus algal biomass (McQueen et al. 1986), TP vesus zooplankton biomass (Pace 1984), and TP versus fish biomass (Downing et al. 1990)). That MEI is a good predictor of nutrient concentrations, yet fails to accurately predict trophic levels above CHL in this study suggests that factors other than nutrients and lake morphometry regulate the standing stock of plankton in lakes (e.g. differences in hydrologic conditions). Future effort should be directed at increasing the sample size and standardizing analytical techniques and sampling times. This study also verified the significant effect of salinity on the MEI–CHL relationship and indicated that relationships should be constructed separately for saline and nonsaline lakes.

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References

BAYLY, I. A. E., AND W. D. WILLIAMS. 1973. Inland waters and their ecology. Longman Australia Pty. Ltd., Victoria. 316 p.

BIERHUIZEN, J. F. H., AND E. E. PREPAS. 1985. Relationship between nutrients, dominant ions, and phytoplankton standing crop in Prairie saline lakes. Can. J. Fish. Aquat. Sci. 42: 1588–1594.

BIRD, D. F., AND J. KALFF. 1984. Empirical relationship between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can. J. Fish. Aquat. Sci. 41: 1015–1023.

CHOW-FRASER, P., AND H. C. DUTHIE. 1987. Response of the phytoplankton community to weekly additions of monoammonium phosphate in a dystrophic lake. Arch. Hydrobiol. 110: 67–82.

CHOW-FRASER, P., AND E. J. MALY. 1991. Factors governing clutch size in two species of *Diaptomus* (Copepoda: Calanoida). Can. J. Fish. Aquat. Sci. 48: 364–370.

CHOW-FRASER, P., AND D. O. TREW. 1990. A compendium of limnological data on 23 lakes in the Beaver River drainage basin. Alberta Environment Technical Report, Environmental Quality Monitoring Branch, Environmental Assessment Division, Edmonton, Alta. 201 p.

DOWNING, J., C. PLANTE, AND S. LALONDE. 1990. Fish production correlated with primary productivity, not the morphoedaphic index. Can. J. Fish. Aquat. Sci. 47: 1929–1936.

FOSTER, I. D. L., I. C. GRIEVE, AND A. D. CHRISTMAS. 1981. The use of specific conductance in studies of natural waters and soil solutions. Hydrol. Sci. Bull. 26: 267–269.

HANSON, J. M., AND W. C. LEGGETT. 1982. Empirical prediction of fish biomass and yield. Can. J. Fish. Aquat. Sci. 39: 257–263.

HENDERSON, H. F., R. A. RYDER, AND A. W. KUDHONGANIA. 1973. Assessing fishery potentials of lakes and reservoirs. J. Fish. Res. Board Can. 30: 2000–2009.

HOYER, M. V., AND J. R. JONES. 1983. Factors affecting the relation between phosphorus and chlorophyll *a* in midwestern reservoirs. Can. J. Fish. Aquat. Sci. 40: 192–199.

JACKSON, D. A., H. H. HARVEY, AND K. M. SOMERS. 1990. Ratios in aquatic sciences: statistical shortcomings with mean depth and the morphoedaphic index. Can. J. Fish. Aquat. Sci. 47: 1788–1795.

JANUS, L. L., AND R. A. VOLLENWEIDER. 1981. The OECD cooperative report on eutrophication: Canadian contribution. Summary Rep. Sci. Ser. No. 132.

JENKINS, R. M. 1982. The morphoedaphic index and reservoir fish production. Trans. Am. Fish. Soc. 111: 133–140.

JONES, J. R., AND M. V. HOYER. 1982. Sportfish harvest predicted by summer chlorophyll-*a* concentration in midwestern lakes and reservoirs. Trans. Am. Fish. Soc. 111: 176–179.

KEREKES, J. 1973. The influence of water renewal on the nutrient supply in small, oligotrophic (Newfoundland) and highly eutrophic (Alberta) Lakes, p. 383–400. *In* E. R. Reinelt et al. [ed.] Proc. Symp. Lakes West. Can. 1972, University of Alberta, Edmonton, Alta.

1975. Phosphorus supply in undisturbed lakes in Kejimkujik National Park, Nova Scotia (Canada). Verh. Int. Ver. Limnol. 19: 349–357.

KERR, S. R. 1982. The role of external analysis in fisheries science. Trans. Am. Fish. Soc. 111: 165–170.

MATUSZEK, J. E. 1978. Empirical predictions of fish yields of large North American lakes. Trans. Am. Fish. Soc. 107: 385–394.

MCQUEEN, D. J., J. R. POST, AND E. L. MILLS. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. 43: 1571–1581.

MURPHY, G. I. 1962. Effect of mixing depth and turbidity on the productivity of fresh water impoundments. Trans. Am. Fish. Soc. 91: 69–76.

NORTHCOTE, T. G., AND P. A. LARKIN. 1956. Indices of productivity in British Columbia lakes. J. Fish. Res. Board Can. 13: 515–540.

NURNBERG, G., AND R. H. PETERS. 1984. Biological availability of soluble reactive phosphorus in anoxic and oxic freshwaters. Can. J. Fish. Aquat. Sci. 41: 757–765.

PACE, M. L. 1984. Zooplankton community structure, but not biomass, influences the phosphorus – chlorophyll *a* relationship. Can. J. Fish. Aquat. Sci. 41: 1089–1096.

PREPAS, E. E. 1983. Total dissolved solids as a predictor of lake biomass and productivity. Can. J. Fish. Aquat. Sci. 40: 92–95.

PREPAS, E. E., AND D. O. TREW. 1983. Evaluation of the phosphorus–chlorophyll relationship for lakes off the Precambrian Shield in western Canada. Can. J. Fish. Aquat. Sci. 40: 27–35.

RANTA, E., AND K. LINDSTROM. 1989. Prediction of lake-specific fish yield. Fish. Res. 8: 113–128.

1990. Water quality versus other determinants of species-specific yield of fish in northern finnish lakes. Fish. Res. 8: 367–379.

RASMUSSEN, J. B., L. GODBOUT, AND M. SCHALLENBERG. 1989. The humic content of lake water and its relationship to watershed and lake morphometry. Limnol. Oceanogr. 34: 1336–1343.

RAWSON, D. S. 1951. The total mineral content of lake waters. Ecology 32: 669–672.

1952. Mean depth and fish production of large lakes. Ecology 33: 513–521.

1960. A limnological comparison of 12 large lakes in northern Saskatchewan. Limnol. Oceanogr. 5: 195–211.

RAWSON, D. S., AND J. E. MOORE. 1944. The saline lakes of Saskatchewan. Can. J. Res. D 22: 141–201.

RYDER, R. A. 1965. A method for estimating the potential fish production of north-temperate lakes. Trans. Am. Fish. Soc. 94: 214–218.

1982. The morphoedaphic index — use, abuse, and fundamental concepts. Trans. Am. Fish. Soc. 111: 154–164.

RYDER, R. A., S. R. KERR, K. H. LOFTUS, AND H. A. REGIER. 1974. The morphoedaphic index, a fish yield estimator — review and evaluation. J. Fish. Res. Board Can. 31: 663–688.

SCHINDLER, D. W., E. J. FEE, AND T. RUSZCYNSKI. 1978. Phosphorus input and its consequences for phytoplankton standing crop and production in the Experimental Lakes Area and in similar lakes. J. Fish. Res. Board Can. 35: 190–196.

SCHLESINGER, D. A., AND H. A. REGIER. 1982. Climatic and morphoedaphic indices of fish yields from natural lakes. Trans. Am. Fish. Soc. 11: 141–150.

SCHNEIDER, D. C., AND R. L. HAEDRICH. 1989. Prediction limits of allometric equations: a reanalysis of Ryder's morphoedaphic index. Can. J. Fish. Aquat. Sci. 46: 503–508.

SHORTREED, K. S., AND J. G. STOCKNER. 1981. Limnological results from the 1979 British Columbia lake enrichment program. Can. Tech. Rep. Fish. Aquat. Sci. 995.

SOKAL, R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman & Co., San Francisco, CA.

STOCKNER, J. G., AND K. S. SHORTREED. 1985. Whole-lake fertilization experiments in coastal British Columbia lakes: empirical relationships between nutrient inputs and phytoplankton biomass and production. Can. J. Fish. Aquat. Sci. 42: 649–658.

STOCKNER, J. G., K. S. SHORTREED, AND K. STEPHENS. 1980. The British Columbia lake fertilization program: limnological results from the first two years of nutrient enrichment. Can. Tech. Rep. Fish. Aquat. Sci. 924.

YOUNGS, W. D., AND D. G. HEIMBUCH. 1982. Another consideration of the morphoedaphic index. Trans. Am. Fish. Soc. 111: 151–153.

ZIMMERMAN, A. P., K. M. NOBLE, M. A. GATES, AND J. E. PALOHEIMO. 1983. Physiochemical typologies of south-central Ontario lakes. Can. J. Fish. Aquat. Sci. 40: 1788–1803.

Gape Limitation and Prey Selection in Larval Yellow Perch (*Perca flavescens*), Freshwater Drum (*Aplodinotus grunniens*), and Black Crappie (*Pomoxis nigromaculatus*)

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We compared prey selection of larval yellow perch (*Perca flavescens*), freshwater drum (*Aplodinotus grunniens*), and black crappie (*Pomoxis nigromaculatus*) in Lake Mendota, Wisconsin. All three species had a diet dominated by copepods and selected progressively larger prey as fish length increased. For a given fish length, freshwater drum selected larger prey and black crappie selected smaller prey than yellow perch. These differences in prey selectivity were partly explainable from differences in gape to length relationships. Freshwater drum did have the largest gape for a given length of the three species, but gape size for black crappie and yellow perch were similar. Gape size predicted 67% of the variability in mean prey size ingested by yellow perch but only 15% for freshwater drum and 8% for black crappie. Although gape size did predict the upper limit of ingestible prey sizes and explained some of the differences in prey selectivity among the three species, both the degree to which the different fishes can ingest prey close to their gape limit and the degree to which gape predicted mean size of ingested prey varied among the three fish species.

Nous avons comparé la sélection des proies par les larves de la perchaude (*Perca flavescens*), du malachigan (*Aplodinotus grunniens*) ainsi que de la marigane noire (*Pomoxis nigromaculatus*) du lac Mendota, Wisconsin. Les trois espèces avaient toutes un régime alimentaire dominé par les copépodes, et elles choisissaient des proies progressivement plus grandes à mesure que les poissons grandissaient. À longueur donnée, le malachigan choisissait des proies plus grandes que celles choisies par la perchaude, alors que la marigane en choisissait des plus petites. Ces différences s'expliquent en partie par des différences dans le rapport entre le tomium et la longueur. Des trois espèces, le malachigan est le poisson qui avait, à une longueur donnée, le rapport le plus élevé du tomium à la longueur, mais la grandeur du tomium était à peu près la même chez la marigane et la perchaude. La grandeur du tomium permettait d'expliquer 67% de la variabilité de la taille moyenne des proies ingérées par la perchaude, mais d'expliquer seulement 15 % de cette variabilité dans le cas du malachigan et de 8 % dans le cas de la marigane. Bien que la largeur du tomium permette de prévoir la limite supérieure de taille des proies ingérables et qu'elle puisse expliquer certaines différences dans la sélectivité des proies par les trois espèces, il demeure que le degré auquel les différents poissons peuvent ingérer des proies dont la taille est voisine de l'ouverture maximale du tomium ainsi que la mesure dans laquelle le tomium permettait de prévoir la taille moyenne des proies ingérées, variait chez les trois espèces.

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The relationships between ontogenetic changes in prey selection by fish larvae and the abundance of zooplankton prey are important for growth and therefore for survival in the first year of a fish's life. An understanding of these relationships is crucial for the study of mechanisms underlying recruitment variability in fish populations (Crowder et al. 1987; Miller et al. 1988; Mills et al. 1989; Shuter and Post 1990). Capture success is influenced by a predator's ability to capture and handle prey (Miller et al. 1988) and morphological constraints, such as gape size. These constraints can be a primary limitation on the kinds of prey ingested by larval fishes (Wankowski 1979; Mathias and Li 1982; Dabrowski et al. 1984; Arts and Evans 1987; Arts and Sprules 1988).

Fish larvae are gape-limited predators. Several studies have documented the effect of gape size on prey selection of larval fish, especially in yellow perch (*Perca flavescens*) (Wong and Ward 1972; Seifert 1972; Hansen and Wahl 1981; Mills et al. 1984; Arts and Evans 1987; Michaletz et al. 1987; Miller et al. 1988). In general, there is a progression towards larger prey as the fish grow and the gape size increases. However, fish larvae may also select particular prey groups within the range they can ingest, and gape size may or may not be a good predictor of prey selection by larval fish. In this study we compare patterns of prey selection by larvae of three fish species, yellow perch, freshwater drum (*Aplodinotus grunniens*), and black crappie (*Pomoxis nigromaculatus*), in Lake Mendota, Wisconsin. We also compare selectivity with changes in gape size occurring during early ontogeny in the three species. Specifically, we address the following questions: (1) are differences in prey selectivity among the three species predictable

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from differences in gape to length relationships, (2) does gape size impose a similar upper limit of ingestible prey in all three species, (3) can gape size be used as a predictor of prey size ingested.

Lake Mendota is a eutrophic lake with a surface area of 39.1 km^2 and maximum depth of 25.3 m (Brock 1985). Currently, a whole-lake food web manipulation is being conducted on the lake (Kitchell 1992). The goal of this manipulation is to introduce large numbers of piscivores, thereby reducing densities of planktivorous fish and increasing grazing by large *Daphnia* to increase water quality (Carpenter et al. 1985; Carpenter et al. 1987). Larval fishes can be abundant and have high specific consumption rates, and their role as predators is rarely quantified (Wong and Ward 1972; Post 1990). An additional objective of the present study was to determine when larvae of major fish species would contribute to *Daphnia* mortality. In Oneida Lake, the collapse of *Daphnia pulex* populations occurred in years of high larval perch biomass (Hansen and Wahl 1981). We hypothesized that there could be a similar effect of larval yellow perch on *Daphnia pulicaria* populations in Lake Mendota.

Materials and Methods

Larval yellow perch, freshwater drum, and black crappie were collected using a fine-mesh purse seine (mesh size 1 mm^2) (Evans and Johannes 1988). The purse seine samples approximately 360 m^3 of water (10 m in diameter and 5.5–6 m deep). Collections were made weekly from May 31 to June 27 and the first and last weeks in July 1989. Samples were collected at five stations, two at the 15-m and two at the 20-m depth contours and one at the deepest site in the lake (25 m). The samples consisted of large amounts of zooplankton (primarily *D. pulicaria*) along with the fish. Fish were separated by hand from the samples and preserved in 95% ethanol. The main species captured in the purse seine throughout the summer were yellow perch, darters (*Etheostoma* spp.), black crappie, freshwater drum, and bluegill (*Lepomis macrochirus*). Details on sampling, fish abundances, and seasonal changes in species composition are in Post et al. (1992). Zooplankton samples were taken within 2 d of the purse seine samples at the 25-m site (Table 1). They were collected using a Clark-Bumpus sampler with 130-μm mesh using oblique tows from 20 m to the surface. Vertical migration of zooplankton in Lake Mendota is limited and the species composition does not change much with depth (Y. C. Allen and C. Luecke, Center for Limnology, University of Wisconsin, Madison, WI 53706, USA, pers. comm.; Dodson 1990). Zooplankton were preserved in 10% formalin, counted, and measured in the laboratory. Details on zooplankton sampling are in Luecke et al. (1992).

The larvae were identified using Auer's (1982) key. When available, 16 yellow perch and freshwater drum and 10 black crappie were selected from each depth contour on each date for further analysis. Fish were chosen to give a uniform distribution over available size groups (Table 2). The total length of each fish below 20 mm was measured with an ocular micrometer to the nearest 0.1 mm. Fish 20 mm and longer were measured to the nearest 0.5 mm with a ruler. Gapes were measured using a gape micrometer built from the description of Arts and Evans (1987). Six different-sized cones with a 20° taper were used interchangeably depending on fish gape. The tip and base diameters mimic those of Arts and Evans (1987). The tips of the cones were aligned with the zeroed micrometer head and placed against a flat microscope slide. The fish were pushed onto the cone until resistance was felt and the flat head plate of the micrometer was adjusted to line up with the edge of the fishes' mouths. This was done under a microscope for more accurate readings. Measurements were then converted into diameters by using the geometric function of a cone.

Contents of the entire digestive tract of each fish were analyzed. Each prey organism was identified using Balcer et al. (1984), counted, and measured. The tip of the helmet to base of the tailspine was measured for *D. pulicaria*. Copepods were measured from the tip of the head to the end of the abdomen. Cladocerans were identified to species. Copepods were separated into two categories, cyclopoids and calanoids, with one calanoid, *Aglodiaptomous clavipes*, identified to species. The copepodites of both calanoids and cyclopoids were grouped together. The few rotifers that were encountered were of the genus *Conochilus*. We used an electivity index suggested by Chesson (1983) to display preference based on the standardized foraging ratio (see Chesson 1983). This index ranges from +1 to −1, with zero indicating no selection. Prey selectivity was calculated separately for each 2-mm fish length group on each sampling occasion.

Results and Discussion

Prey Selection

The zooplankton size composition remained relatively constant over the purse seine dates with the exception of an

TABLE 1. Range and mean length of fish larvae used in the analysis and absolute densities of each of the four prey size categories. Zooplankton samples were taken on the following dates: June 1, 7, 14, 21, and 29, July 4 and 26, and August 1.

Date (1989)	Species	Size range (mm)	*N*	Mean size (mm)	Prey density (no./L) for each size category (mm)			
					0–0.25	0.25–0.5	0.5–1.0	1.0–2.5
May 31	Yellow perch	8.1–15.6	80	12.3	7.59	4.83	12.18	15.33
June 8	Yellow perch	10.8–23.0	64	15.8	9.46	11.46	10.23	15.72
June 12	Yellow perch	12.8–21.0	80	16.9	13.32	11.19	7.61	16.11
June 20	Yellow perch	16.1–23.5	58	19.9	5.63	6.4	4.2	7.1
	Black crappie	5.9–13.6	23	9.0				
June 27	Black crappie	9.9–18.4	34	13.7	3.3	4.96	6.51	13.57
	Freshwater drum	5.8–8.9	5	7.2				
July 6	Black crappie	8.4–16.7	39	12.1	6.04	4.38	13.275	9.75
	Freshwater drum	4.3–14.9	72	9.5				
July 24	Black crappie	9.4–16.1	37	12.5	13.91	12.96	17.752	2.45
	Freshwater drum	6.9–16.4	51	12.2				
August 1	Black crappie	11.4–17.4	29	14.4	7.85	6.36	11.63	3.72

TABLE 2. Least-square linear regression equations for gape diameter (G, mm) as a function of fish length (L, mm) for the three species. SE is the standard error. The equations for yellow perch and black crappie are not significantly different.

Species	Equation	SE intercept	SE slope	Size range (mm)	r^2	N
Yellow perch	$G=0.159L-0.597$	0.039	0.002	8.1–23.5	0.94	287
Freshwater drum	$G=0.175L-0.228$	0.049	0.004	4.3–16.4	0.92	132
Black crappie	$G=0.161L-0.656$	0.093	0.007	5.9–18.4	0.75	162

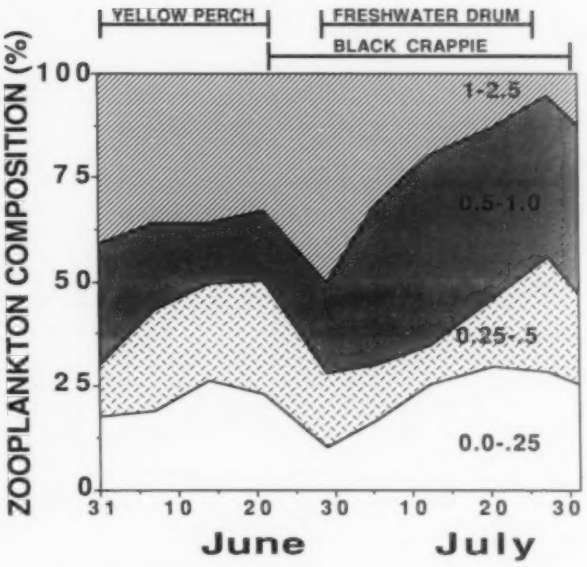

FIG. 1. Zooplankton size composition throughout the purse seine sampling season, May 31 to August 1. Prey size classes correspond to taxonomic groupings as follows: 0.0–0.25 mm, nauplii; 0.25–0.50 mm, copepodites; 0.5–1.0 mm, adult copepods (except *A. clavipes*); 1.0–2.5 mm, immature and adult *D. pulicaria* and *A. clavipes*. Zooplankton abundance decreased from a total of 50 to 25 individuals per litre during this period. Absolute densities of each prey size category are given in Table 1. The upper bars represent the time of year larvae of each fish species occurred in the purse seine samples.

increase in the largest size class in the beginning of July and a decrease at the end of July (Fig. 1). Zooplankton densities decreased from a total of 50 to 25 individuals per litre during this time period. Luecke et al. (1992) give detailed information on the seasonal dynamics of zooplankton in Lake Mendota. Yellow perch larvae were caught between May 31 and June 20, black crappie between June 20 and July 31, and freshwater drum between June 27 and July 24. Thus, the catch of freshwater drum and black crappie overlapped in time whereas yellow perch were caught earlier in the season (Fig. 1; see also Post et al. 1992). The size ranges of fish studied were 8–23.5 mm for yellow perch, 6–18 mm for black crappie, an 4–16 mm for freshwater drum. Table 1 shows the size ranges of fish collected by date along with absolute densities of each of the four size categories of prey.

Yellow perch showed a progression in prey selection with increasing fish size (Fig. 2). Only the smallest perch (8 mm) selected nauplii (0–0.25 mm). As the perch increased in size, first copepod copepodites (0.25–0.5 mm), then adult copepods (0.5–1 mm), and finally larger prey 1–2.5 mm) became the most selected prey size. Sampling date had little effect compared with that of fish size. This trend of prey size selectivity corresponded to observations on yellow perch by other investigators (Wong and Ward 1972; Seifert 1972; Hansen and Wahl 1981; Mills et al. 1984; Confer and O'Bryan 1989). Freshwater drum showed a similar pattern of increased selection of larger prey with an increase in length, but selected larger prey sizes at a smaller fish length than the other two species (Fig. 2). Freshwater drum had a relatively consistent positive selection for the 0.5–1 mm prey items (adult copepods) and a negative selection for nauplii. The selection of 0.25–0.5 mm prey items decreased as the selection of the largest size class (1–2.5 mm) increased. Black crappie selected prey sizes 0.25–0.5 m and 0.5–1 mm to a larger extent than the freshwater drum. In contrast with the other two species, black crappie sometimes selected nauplii at a relatively large fish size. Although the selectivity patterns for black crappie were more variable than those of the other two fishes, they also showed a tendency towards an increased selectivity for larger items as fish length increased (Fig. 2).

Gape Limitation

We hypothesized that the observed differences in prey selectivity among the three species could be explained by differences in gape to length relationships. Because freshwater drum fed on the largest items and black crappie on smallest items for a given fish length, we expected gape to be largest in freshwater drum, followed by yellow perch and black crappie. This was only partly true. Freshwater drum had a larger gape diameter for a given fish length than both yellow perch and black crappie (Fig. 3; Table 2). However, the gape to length relationships for yellow perch and black crappie were similar and neither the slope nor the intercept was significantly different from both black crappie and yellow perch ($P = 0.48$, intercept). The intercept of freshwater drum was significantly different from both black crappie and yellow perch ($P = 0.0083$, slope; $P = 0.0001$, intercept). The regression equation found for yellow perch was similar to the one presented by Arts and Evans (1987) (obtained with identical methods), but different from the one reported by Michaletz et al. (1987) (using vertical opening as measure of gape). Freshwater drum have one of the largest gape to body size relationships reported to date with the exception of the regression calculated for walleye (*Stizostedion vitreum vitreum*) by Mathias and Li (1982), using the same method as Michaletz et al. (1987)

The size of prey found in the stomach was, with few exceptions, smaller than the maximum gape size in all fish (Fig. 4). However, gape appeared to be more limiting to yellow perch than to freshwater drum and black crappie. Freshwater drum tended to ingest prey as big as their gape size. This was also true initially for black crappie, but they continued to feed on smaller items even when their ability to ingest larger prey

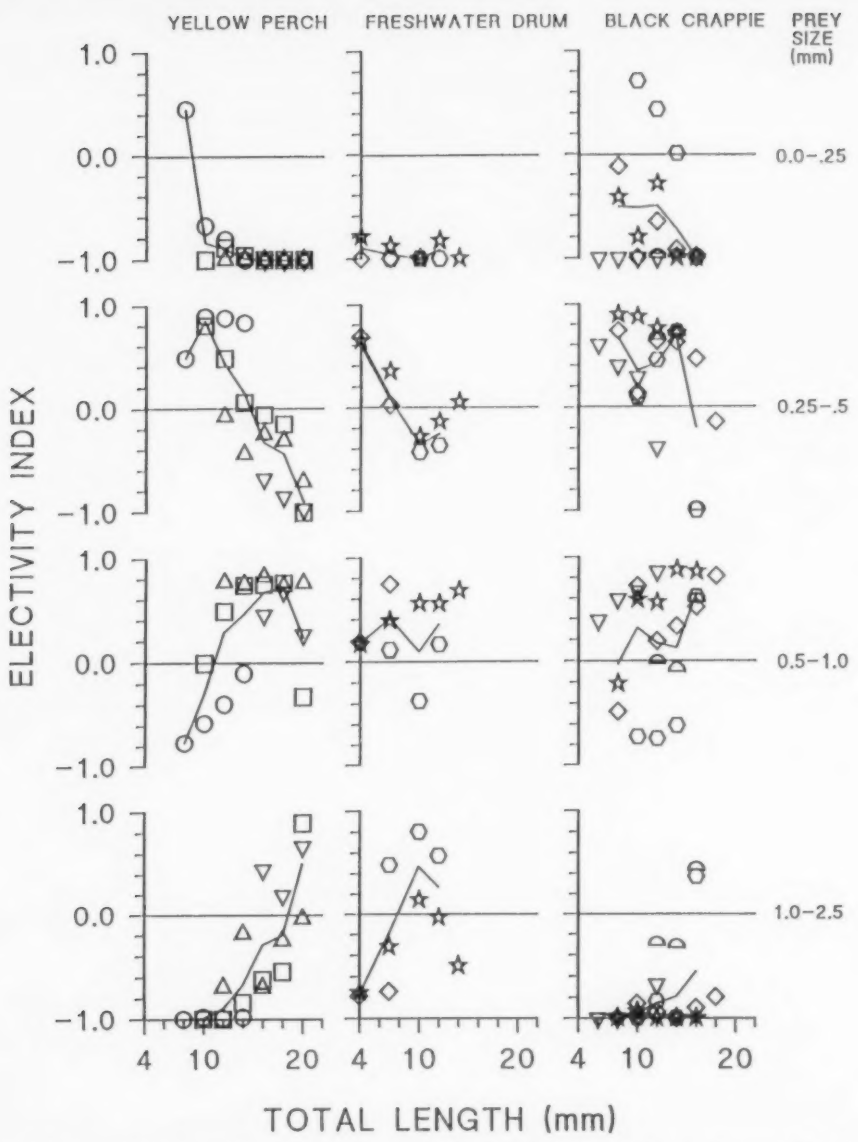

FIG. 2. Prey selection as a function of fish size for larval yellow perch, freshwater drum, and black crappie. The electivity index is based on the standardized foraging ratio (see Materials and Methods). Electivities are calculated for each 2-mm fish size group and each date. The means for each fish size class — are connected. Only data based on five or more fish larvae are included. The different symbols represent the different sampling dates: ○, May 31; □, June 8; △, June 12; ▽, June 20; ◇, June 27; ☆, July 6; ⬡, July 24; ⌂, August 1.

increased. To test this, we calculated a ratio between prey size and gape size for all prey consumed. The largest 5% of these ratios was significantly different among the three species, with the smallest values for yellow perch and largest for freshwater drum (Wilcoxon signed ranks, using only fish 8–16 mm, the size group caught in all three species.)

Yellow perch, freshwater drum, and black crappie feed on smaller zooplankton sizes than the average size available in the lake even though their gape is large enough to allow ingestion of average size zooplankton. This is consistent with most other reports (Wong and Ward 1972; Hansen and Wahl 1981; Mills et al. 1984; Post and McQueen 1988; Confer and O'Bryan 1989; Ponton and Müller 1990; but see Michaletz et al. 1987). Wong and Ward (1972) attributed this to "gut packing." Smaller prey may be selected to increase prey density in the digestive tract. This seems unlikely because the number of prey items in the stomachs of fish of this size was generally low. However, digestion of larger prey items is thought to be more difficult than digestion of intermediate-sized prey (Mills et al. 1984; Confer and Lake 1987; Confer et al. 1990). The fish may

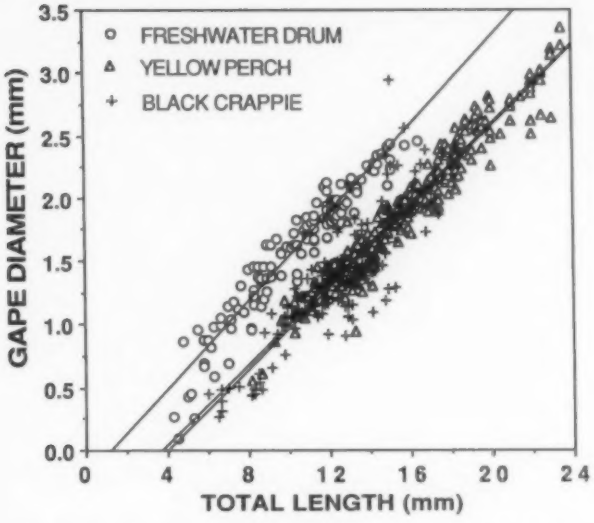

FIG. 3. Gape to length relationship for larval yellow perch, freshwater drum, and black crappie. Equations are given in Table 2. The equations for yellow perch and black crappie are not significantly different.

also learn to utilize familiar prey size classes through experience (Hansen and Wahl 1981), which would contribute to a continuing selection of smaller prey sizes even when larger prey can be ingested.

Maximum gape diameter sets the upper limit for ingestible prey size in all three species. Yellow perch selected progressively larger prey items as they grew, and for this species, gape was also a good predictor of the mean size for ingested prey. This was less true for freshwater drum and black crappie (Fig. 5). Gape size explained only 15 and 8% of the variation in average prey size eaten for freshwater drum and black crappie, respectively. This is partly attributable to the low number of prey larger than 1 mm available on July 24 and August 1, but large black crappie and freshwater drum were included in the analysis from June 27 and July 6 when the density of prey larger than 1 mm was higher (Table 1). If the pattern of different prey availability was caused by large prey not being in the water column, then both the black crappie and the freshwater drum would have been affected. The low number of large prey in black crappie and freshwater drum is not due to changes in the taxonomic composition of the largest prey class. The largest copepod (*A. clavipes*) was more selected than *D. pulicaria* by all three fish species, and this copepod was rare in the lake samples, only increasing slightly at the end of July. Also, black crappie on average fed on smaller prey than yellow perch at the same gape size. Thus, the relationship between gape size, fish size, and prey selection for yellow perch is not directly transferable to other species.

Application to the Food Web Manipulation Experiment

One goal of the food web manipulation project in Lake Mendota is to decrease planktivory on large *Daphnia*, since these zooplankton are the main grazers on algae in the lake and large *Daphnia* appear to be necessary for improving water quality through increased grazing on phytoplankton (McQueen et al. 1986; Carpenter et al. 1987). High abundances of young-of-the-year yellow perch fry have been correlated with a decrease

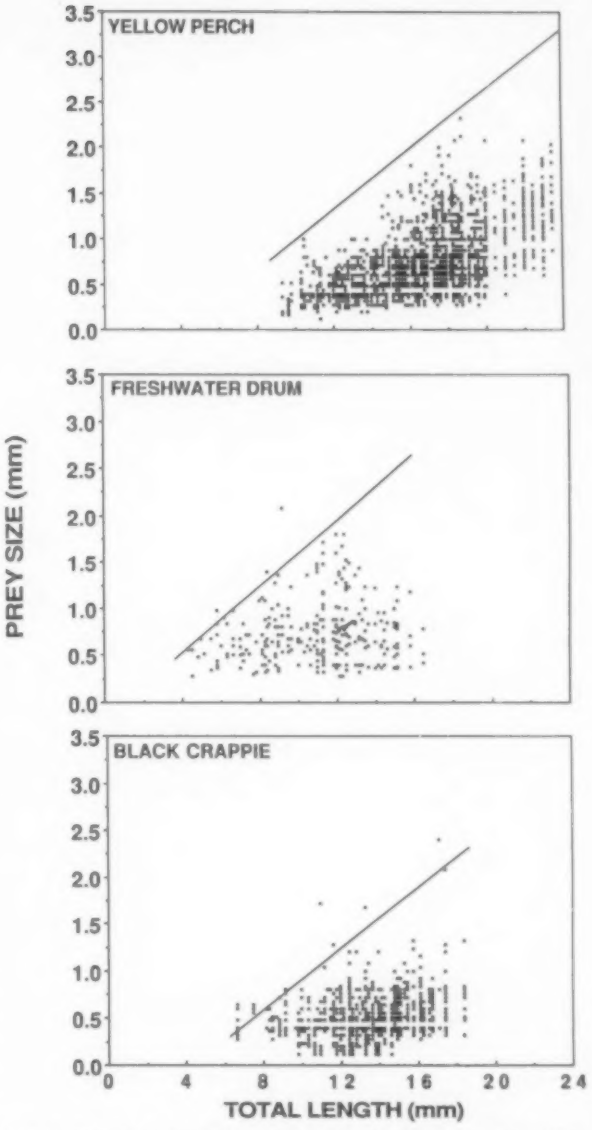

FIG. 4. Size of individual prey plotted against fish size for yellow perch, freshwater drum, and black crappie. The lines represent the change in gape size as a function of fish size from Fig. 3. Each point represents one prey item, and one fish may contribute up to 20 prey items.

in the *D. pulex* population in Oneida Lake (Hansen and Wahl 1981). However, none of the three species of larval fish investigated here selected the large *D. pulicaria* over copepods in Lake Mendota. This is probably a result of the small size of the fish larvae in this study. Even though the gape diameter of yellow perch should allow them to feed on *Daphnia* at 16 mm length, they do not select *Daphnia*-sized prey until they are larger than 20 mm. Other investigators who have reported that yellow perch fry select the larger *D. pulex* worked on larger fish (20–50 mm, Wong and Ward 1972; 18–60 mm, Hansen and Wahl 1981; 18–60 mm, Mills et al. 1984; 30–60 mm, Mills et al. 1989). Hansen and Wahl (1981) concluded that yellow

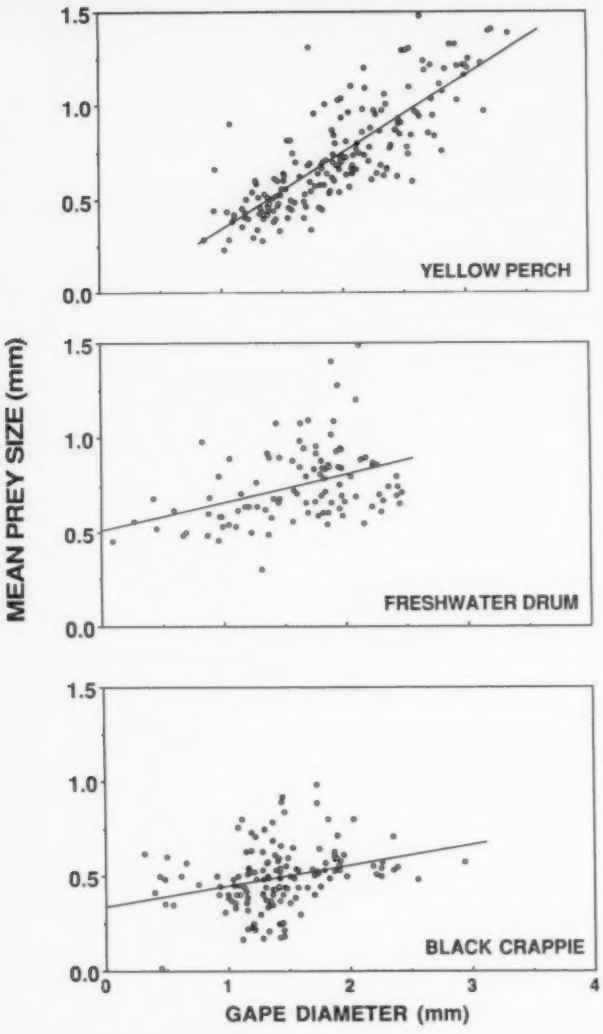

FIG. 5. Mean size of prey eaten by an individual fish as a function of its gape diameter for yellow perch, freshwater drum, and black crappie. Regression equations are as follows: yellow perch, $y = 0.41x - 0.007$, $r^2 = 0.67$, $N = 176$; freshwater drum, $y = 0.05 + 0.15x$, $r^2 = 0.15$, $N = 102$; black crappie, $y = 0.11x + 0.33$, $r^2 = 0.08$, $N = 146$.

perch are selective for *D. pulex* only after they reach 28 mm in length. A recent laboratory experiment by Confer et al. (1990), which supported earlier work (Confer and O'Bryan 1989), showed that smaller *D. pulex* and copepods (especially calanoids) were selected by yellow perch larvae, even when the densities of large *D. pulex* were high and the fish were morphologically able to ingest larger prey. Since larger yellow perch (≥25 mm) and centrarchid fry (≥20 mm) move to the littoral zone in Lake Mendota, we expect little effect of young-of-the-year fishes on the open-water *Daphnia* population in this lake (Post et al. 1992).

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References

ARTS, M. T., AND D. O. EVANS. 1987. Precision micrometer measurement of mouth gape of larval fish. Can. J. Fish. Aquat. Sci. 44: 1786–1791.

ARTS, M. T., AND W. G. SPRULES. 1988. Large daphnia as an indicator of fish predation on another zooplankton species (*Holopedium gibberum* ZADDACH). Verh. Int. Ver. Limnol. 23: 1685–1691.

AUER, N. A. 1982. Identification of larval fishes of the great lakes basin with emphasis on the Lake Michigan drainage. Great Lakes Fish. Comm. Spec. Publ. 82–3.

BALCER, M. D., N. L. KORDA, AND S. I. DODSON. 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. The University of Wisconsin Press, Madison, WI. p. 26–48.

BROCK, T. D. 1985. A eutrophic lake: Lake Mendota, Wisconsin. Springer-Verlag, New York. NY. p. 16, 194–197.

CARPENTER, S. R., J. F. KITCHELL, AND J. R. HODGSON. 1985. Cascading trophic interactions and lake productivity: fish predation and herbivory can regulate lake ecosystems. Bioscience 35: 634–639.

CARPENTER, S. R., J. F. KITCHELL, J. R. HODGSON, P. A. COCHRAN, J. J. ELSER, M. M. ELSER, D. M. LODGE, D. KRETCHMER, X. HE, AND C. N. VONENDE. 1987. Regulation of lake primary productivity of food web structure. Ecology 68: 1863–1876.

CHESSON, J. 1983. The estimation and analysis of preference and its relationship to foraging models. Ecology 64(5): 1297–1304.

CONFER, J. L., AND G. J. LAKE. 1987. Influence of prey type on growth of young yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 44: 2028–2033.

CONFER, J. L., E. L. MILLS, AND L. O'BRYAN. 1990. Influence of prey abundance in species and size selection by young perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 47: 882–887.

CONFER, J. L., AND L. O'BRYAN. 1989. Changes in prey rank and preference by young planktivores for short-term and long-term ingestion periods. Can. J. Fish. Aquat. Sci. 46: 1026–1032.

CROWDER, L. B., M. E. MCDONALD, AND J. A. RICE. 1987. Understanding recruitment of Lake Michigan fishes: the importance of size-based interactions between fish and zooplankton. Can. J. Fish. Aquat. Sci. 44(Suppl. 2): 141–147.

DABROWSKI, K. R., E. MURAWSKA, J. TERLECKI, AND S. WIELGOSZ. 1984. Studies on the feeding of *Coregonus pollan* (Thompson) alevins and fry in Lough Neagh. Int. Rev. Hydrobiol. 69: 529–541.

DODSON, S. I. 1990. Predicting diel vertical migration of zooplankton. Limnol. Oceanogr. 35: 1195–1200.

EVANS. D. O., AND P. R. JOHANNES. 198. A bridle-less trawl and fine-mesh purse seine for sampling pelagic coregonine larvae with observations of spatial distribution and abundance. Ont. Fish. Tech. Rep. No. 27: 1–19.

HANSEN, M. J., AND D. H. WAHL. 1981. Selection of small *Daphnia pulex* by yellow perch fry in Oneida Lake, New York. Trans. Am. Fish. Soc. 11: 64–71.

KITCHELL, J. F. [ED.] 1992. Food web research and its application to lake management: a case study of Lake Mendota, Wisconsin. Springer-Verlag. New York, NY. (In press).

LUECKE, C., L. G. RUDSTAM, AND Y. C. ALLEN. 1992. Interannual patterns of planktivory 1987–1989: an analysis of vertebrate and invertebrate planktivores. *In* J. F. Kitchell [ed.] Food web research and its application to lake management: a case study of Lake Mendota, Wisconsin. Springer-Verlag, New York, NY. (In press)

MATHIAS, J. A., AND S. LI. 1982. Feeding habits of walleye larvae and juveniles: comparative laboratory and field studies. Trans. Am. Fish. Soc. 111: 722–735.

MCQUEEN, D. J., J. R. POST, AND E. L. MILLS. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. 38: 1571–1581.

MICHALETZ, P. H., D. G. UNKENHOLZ, AND C. C. STONE. 1987. Prey size selectivity and food partitioning among zooplanktivorous age-0 fishes in lake Francis Case, South Dakota. Am. Mid. Nat. 117(1): 126–138.

MILLER, T. L., L. B. CROWDER, J. A. RICE, AND E. A. MARSCHALL. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. 45: 1657–1670.

MILLS, E. L., J. L. CONFER, AND R. C. READY. 1984. Prey selection by young yellow perch: the influence of capture success, visual acuity and prey choice. Trans. Am. Fish. Soc. 113: 579–587.

MILLS, E. L., R. SHERMAN, AND D. S. ROBSON. 1989. Effect of zooplankton abundance and body size on growth of age-0 yellow perch (*Perca flavescens*) in Oneida Lake, New York, 1975–86. Can. J. Fish. Aquat. Sci. 46: 880–886.

PONTON, D., AND R. MÜLLER. 1990. Size of prey ingested by whitefish, *Coregonus* sp., larvae. Are *Coregonus* larvae gape-limited predators? J. Fish. Biol. 36: 67–72.

POST, J. R. 1990. Metabolic allometry of larval and juvenile yellow perch (*Perca flavescens*): in situ estimates and bioenergetic models. Can. J. Fish. Aquat. Sci. 47: 554–560.

POST, J. R., AND D. J. MCQUEEN. 1988. Ontogenetic changes in the distribution of larval and juvenile yellow perch (*Perca flavescens*): response to prey or predators? Can. J. Fish. Aquat. Sci. 45: 1820–1826.

POST, J. R., L. G. RUDSTAM, D. M. SCHAEL, AND C. LUECKE. 1992. An energetics based assessment of the potential impact of pelagic larval and juvenile fish on Lake Mendota zooplankton community composition. *In* J. F. Kitchell [ed.] Food web research and its application to lake management: a case study of Lake Mendota, Wisconsin. Springer-Verlag, New York, NY. (In press)

SEIFERT, R. L. 1972. First food of larval yellow perch, white sucker, bluegill, emerald shiner, and rainbow smelt. Trans. Am. Fish. Soc. 101: 219–225.

SHUTER, D., AND J. R. POST. 1990. Climate, population viability and zoogeography of temperate fishes. Trans. Am. Fish. Soc. 119: 314–336.

WANKOWSKI, J. W. J. 1979. Morphological limitations, prey size selectivity, and growth response of juvenile Atlantic salmon, *Salmo salar*. J. Fish Biol. 14: 89–100.

WONG, B., AND F. J. WARD. 1972. Size selection of *Daphnia pulicaria* by yellow perch (*Perca flavescens*) fry in West Blue Lake, Manitoba. J. Fish. Res. Board Can. 29: 1761–1764.

Drift Responses of Benthic Invertebrates to Experimental Streamflow Variation in a Hydrologically Stable Stream

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Poff, N. L., and J. V. Ward. 1991. Drift responses of benthic invertebrates to experimental streamflow variation in a hydrologically stable stream. Can. J. Fish. Aquat. Sci. 48: 1926–1936.

Field experiments were conducted in the regulated upper Colorado River to assess drift responses of lotic macroinvertebrates to streamflow manipulations. In each of three seasons, drift was collected in one control and two experimental riffles. On the first day, no flow manipulations occurred. Six hours before sunset on the second day, streamflow was simultaneously reduced and elevated in two experimental riffles with instream diversion structures. Following flow elevation, both mean daily drift density and drift rate generally increased for 13 taxa across all seasons. Flow reductions generally induced elevated drift densities for most taxa, but drift rates declined for some taxa. Patterns of diel drift periodicity were less frequently modified by flow manipulations. Taxa with typical nocturnal peaks in drift activity (*Baetis* spp., *Epeorus longimanus*, *Triznaka signata*) generally maintained this pattern despite some increases in diurnal drift. For a few taxa, modification of diel drift patterns occurred, either as nocturnal decreases following reduced flow (*Paraleptophlebia heteronea*, *Ephemerella infrequens*) or as diurnal drift increases in response to either elevated flow (*Lepidostoma ormea*, Chironomidae larvae) or reduced flow (Simuliidae). With some exceptions, observed drift responses could be used to suggest active versus passive processes of drift entry.

On a procédé à des expériences sur le terrain dans la partie amont du fleuve Colorado, qui est régularisée, afin d'évaluer de quelle façon les changements de débit affectent la dérive des macroinvertébrés lotiques. Chacune des trois saisons où ces expériences ont été effectuées, on a relevé les caractéristiques de dérive dans un seuil témoin et deux seuils expérimentaux. Le premier jour, le débit n'a pas été modifié. Le deuxième jour, 6 h avant le coucher du soleil, on a simultanément réduit et accéléré le débit dans les deux seuils expérimentaux au moyen de structures installées dans le cours d'eau. Suite à l'augmentation de débit, la moyenne quotidienne à la fois du taux de dérive et de la densité d'organismes dérivants s'est élevée de façon générale pour 13 taxons pour l'ensemble des saisons. Les réductions de débit ont normalement provoqué une augmentation de la densité d'organismes dérivants pour la plupart des taxons mais les taux de dérive ont baissé pour certains d'entre eux. Sur la base d'une journée complète, la répartition de la périodicité de la dérive était moins fréquemment modifiée par les manipulations du débit. Les taxons qui se caractérisaient typiquement par des pointes de dérive durant la nuit (*Baetis* sp., *Epeorus longimanus*, *Triznaka signata*) ne modifiaient généralement pas ce comportement algré un certain accroissement de leur dérive nocturne. Pour quelques taxons, il s'est produit une modification de la répartition des périodes de dérive sur la base d'une journée complète, cette modification se traduisant soit par une diminution de la dérive nocturne à la suite de la réduction du débit (*Paraleptophlebia heteronea*, *Ephemerella infrequens*), soit par une augmentation de la dérive diurne consécutivement dans un cas à un accroissement du débit (*Lepidostoma ormea*, larves de chironomidés), et dans un autre à une réduction du débit (Simuliidae). Avec quelques exceptions, il s'est avéré possible de se fonder sur les caractéristiques de dérive observées dans le cadre de ces expériences pour déterminer si celle-ci s'opère de manière active ou passive.

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The downstream transport of benthic invertebrates in the water column (drift) has been related to a variety of biotic and abiotic variables, including rate of water movement (see reviews by Waters 1972; Statzner et al. 1984; Brittain and Eikeland 1988). Rapid changes in streamflow often induce increases in drift rate (organisms per time) or drift density (organisms per volume). Increased drift may result both from flow increases (Elliott 1968; Anderson and Lehmkuhl 1968; Pearson and Franklin 1968; McLay 1968; Brooker and Hemsworth 1978; Crisp and Robson 1979; Scullion and Sinton 1983; Irvine and Henriques 1984; Irvine 1985; Perry and Perry 1986; Graesser 1988; Hooper and Ottey 1988) and from flow decreases (Minshall and Winger 1968; Pearson and Franklin 1968; Radford and Hartland-Rowe 1971; Gore 1977; Corrarino and Brusven 1983; Hooper and Ottey 1988). Changes in streamflow can modify microhabitat characteristics and thus potentially alter diel activity and drift patterns of benthic invertebrates. One obvious ecological implication is the possibility that invertivorous fishes might benefit from increased drift densities (e.g. Elliott 1973).

Differential drift responses of invertebrates to increased versus decreased flow have received limited attention, in part due to natural hydrologic variability characteristic of most natural streams. Most field studies of drift responses to varying discharge have occurred when the investigator(s) had no control over hydrologic conditions. Some experimental manipulations

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of flow have been performed in laboratory streams (e.g. Corkum et al. 1977; Ciborowski 1983; Wilzbach et al. 1988) and in the field, both in streamside channels (Corrarino and Brusven 1983; Irvine 1985; Irvine and Scott 1988) and in natural channels (Hinckley and Kennedy 1972; Irvine and Henriques 1984; Hooper and Ottey 1988). However, previous field experiments on differential drift response to increased versus decreased flow have often confounded treatment effects by inducing sequential flow fluctuations at the same location over one to a few diel cycles, such that organism responses (drift) to separate events are not independent (e.g. Hinckley and Kennedy 1972; Irvine and Henriques 1984; Hooper and Ottey 1988).

Altered flow patterns often increase drift densities by enhancing nocturnal peaks of abundance (e.g. Perry and Perry 1986). The frequently observed nocturnal peak in drift density exhibited by many drifting organisms (Waters 1962) has been interpreted as a proximate consequence of negative phototaxis (e.g. Elliott 1968) and as an evolutionary adaptation to avoidance of visually cued, day-active fish predators (Allan 1978). Considerable interest remains as to whether drift entry is a consequence of passive (i.e. accidental dislodgment) or active (e.g. foraging behavior) processes (see Wilzbach 1990). Insofar as the relative contributions of these two processes are influenced by stream discharge, simultaneous daytime increases and decreases in streamflow might generate drift patterns that could distinguish between these processes.

In the regulated upper Colorado River, a highly regular and stable seasonal flow pattern allowed us to investigate experimentally how local populations of various invertebrate taxa responded to simultaneous increases and decreases in flow. By independently increasing and decreasing flow during daylight hours in separate riffles over three seasons, and by comparing the diel drift responses with an unmanipulated reference riffle, we examined the following questions for a variety of taxa: (1) Do diurnal flow alterations modify mean drift density, drift rate, or diel periodicity of drift? (2) Do changes in drift density, drift rate, or diel pattern depend on direction of flow change? (3) Are there seasonal differences in drift patterns relative to direction of flow modification? (4) What inferences, if any, can be made concerning active versus passive drift entry by comparing differential drift responses with simultaneous increases and decreases in flow?

Methods

Study Site and Experimental Riffles

The study was conducted in the upper Colorado River (2420 m elevation), 7 km below Granby Reservoir, a hypolimnetic-release storage impoundment (~105°55′W, 40°07′N). The thermal regime at the site was not influenced by regulation (cf. Rader and Ward 1988), but the flow regime was entirely governed by reservoir discharge. Mean daily discharges in the stream for the summer, autumn, and spring sampling periods (see below) were 1.16, 0.57, and 2.09 $m^3 \cdot s^{-1}$, respectively (U.S. Bureau of Reclamation, unpubl. data). At the time of the summer sampling, flow in the river had ranged from 1.13 to 1.58 $m^3 \cdot s^{-1}$ for the immediately preceding 26 d. In the autumn, flow had been a constant 0.51–0.62 $m^3 \cdot s^{-1}$ for the previous 71 d. Prior to spring sampling, discharge was slightly more variable and had ranged from 1.98 to 2.26 and from 1.98 to 2.55 $m^3 \cdot s^{-1}$ for the preceding 8 and 36 d, respectively.

Three riffles, spaced over a ~250 m reach, were selected for the experiments. Each riffle was longitudinally partitioned at midstream by attaching corrugated metal roofing sheets (7 m in length) to metal fence posts that had been driven into the substratum. For each riffle, one half of the divided stream was designated the experimental channel. The experimental channel furthest upstream served as an unmanipulated reference site (hereafter referred to as the "reference riffle"). The experimental channel furthest downstream was modified by extending fence posts from the upstream end of the sheet-metal divider to one stream bank. Placement of boards along this extension effectively created a wing dam that diverted water from the entire stream channel through the experimental channel ("high-flow riffle"). The remaining experimental channel (75 m upstream of the high-flow riffle and 150 m downstream of the reference riffle) was also modified with a wing dam, but one that could be used to decrease flow in the channel by diverting flow away from the experimental channel ("low-flow riffle"). We view 75 m as sufficient separation to measure independent drift responses because it exceeds the distance reportedly drifted by invertebrates in one diel cycle under noncatastrophic conditions (see Brittain and Eikeland 1988).

Substratum in the riffles was composed of cobbles, boulders, and interspersed smaller particles, including sand. Other characteristics of the experimental riffles are summarized in Table 1.

Drift Collections and Flow Manipulations

Drift was collected at the downstream end of each experimental channel over two consecutive diel periods in each of three seasons (summer: 27–28 August 1985; autumn: 15–16 November 1985; spring: 5–6 June 1986). For each of the two sampling days in a given season, the same times of day were sampled in all riffles. Drift collections were made at 3-h intervals, seasonally adjusted so that the first sample was taken 6 h before sunset and the third sample was taken 20 min after the sun disappeared behind the horizon (sunset in summer = 20:00, in autumn = 17:00, and in spring = 21:00). The diurnal and nocturnal collections were considered to sample adequately the diel response of drifting invertebrates (see Allan and Russek 1985). An additional predawn sample (05:00) was taken in summer only. Also in summer, the first daytime sample was lost. Sampling in autumn and spring occurred during the absence of moonlight. In summer, bright moonlight occurred during the first night but not during the second night of sampling.

Three drift nets were used for each sampling time in each riffle. Nets were 45.7 cm wide, 30.5 cm high, and 100 cm long and were mounted on steel stakes driven permanently into the substratum for the duration of the study. A 240-μm net was always positioned between two 425-μm nets, with at least 30 cm separating adjacent nets. During each 2-d seasonal sampling period, nets remained in the water for the same amount of time (1 h in summer and autumn, 30 min in spring).

Experimental flow alteration followed the same procedure in each of the three sampling seasons. On the first day of each 2-d sampling period, flow was not manipulated in any riffle. This allowed the premanipulation drift pattern to be established for each of the four sampling times in each riffle. On the second day, the wing dams in the low- and high-flow riffles were put in place immediately prior to the first diurnal sampling time

(i.e. 6 h before sunset). The altered flow regimes were maintained through the last nighttime samples.

Quantity of water filtered by each net in each riffle was determined by multiplying net mouth area times average velocity times sampling duration. In each riffle, velocity readings (Pygmy-Gurly meter) were taken at 0.5 stream depth in the center and at the sides of each net. Measurements were taken immediately after nets were placed in the steam and at the end of the 0.5- or 1-h sampling period. The six values per net were then combined to provide average velocity per net. These measurements were made only once per day because whole-channel discharge did not change during any 2-d period. In the manipulated riffles, measurements were made on day 1 (before manipulation) and day 2 (after manipulation) whereas in the reference riffle, measurements were taken only on day 1. The depth and velocity measurements taken for the three nets in a riffle were used to describe mean riffle depth and velocity.

Statistics

Whole-riffle experimental units were not true, spatial replicates (see Hurlbert 1984), but temporal control data (i.e. pre- and post-manipulation data for all times in all riffles) allow inference of treatment effects. Between-day differences that were observed in the manipulated riffle(s), but not in the unmanipulated reference riffle, can be attributed to the flow manipulations (see discussions in Stewart-Oaten et al. 1986; Carpenter et al. 1988; Carpenter 1990).

Changes in drift density

For each riffle, the *difference* between drift density (number per cubic metre) on day 1 and day 2 in each of three nets was determined for each daily sampling period. Between-day differences were always calculated for nets occupying the same position in a riffle on consecutive days. Thus for each season, riffle, and sampling time, there were three replicates (i.e. between-day differences for matched nets). A square root transformation was applied to produce homogeneity of variance.

Two types of statistical comparisons were performed using SPSSx: within-riffle and among-riffle. For differences *within* each riffle, an AOV ($F_{1,6}$) was used to test for significant changes in mean drift density between days (H_0: between-day difference = 0). Significant differences in the reference riffle would suggest a "day effect" that would be confounded with any flow-induced differences in the manipulated riffles. Both parametric tests and graphical display of the mean ± SE were used to evaluate results (cf. Carpenter et al. 1988). To test for drift differences *among* riffles attributable to the flow manipulations, a one-way AOV ($F_{2,6}$) was used to compare riffles at each of the four sampling times (H_o: between-day difference in manipulated riffle = difference in reference riffle). For each sampling time, a Student–Newman–Keuls pairwise comparison (with df adjusted downward to reflect unequal variances across riffles) was used to test the null hypothesis (Steel and Torrie 1980).

Changes in daily drift rate

Total numbers of organisms drifting were determined for each sampling date in each riffle by summing across all sampling times. Drift rates are unadjusted for stream velocity, which can affect drift distance (e.g. Elliott 1971) and thus influence drift density. Also, drift rates provide a separate indication of drift responses to flow manipulations because the number of drifting animals is not adjusted by streamflow, the experimental treatment variable. Between-day differences for total daily numbers (untransformed) were calculated for each riffle in each season.

Changes in diel drift periodicity

Diel drift patterns for each taxon were examined by determining mean diurnal and mean nocturnal drift density for each sampling day and calculating what proportion of the total drift occurred at night (i.e. nocturnal/(nocturnal + diurnal)). In the summer, only samples from the first two nighttime periods were used in determining nocturnal drift density. Between-day changes in this ratio for the two manipulated riffles were graphically compared with any changes in the reference riffle to assess flow-induced modification of diel periodicity.

Results

In all seasons, mean depth, current velocity, and hence flow decreased in the low-flow riffle and increased in the high-flow riffle following flow manipulation (Table 1). In autumn, flow in the Colorado River was only ~0.6 $m^3 \cdot s^{-1}$ and substantial experimental reduction was not possible, as reflected in Table 1.

Changes in Drift Density

More than 95% of all organisms in the drift consisted of 14 taxa of Ephemeroptera, Plecoptera, Trichoptera, and Diptera. Between-day differences in drift densities for these taxa were more frequent and of greater magnitude in the manipulated riffles than in the reference riffle (see Table 2), thus supporting the inference that flow manipulations were primarily responsible for the observed patterns. In the unmanipulated reference riffle, there was a significant ($p < 0.1$) between-day difference in only 4/33 instances (i.e. combined across seasons). In the low-flow riffle, mean between-day drift densities were significantly different in 19/33 cases across seasons, thereby indicating a strong response to reduced flow. In 15 of these 19 instances, mean daily drift density increased after flow reduction. Similarly, in the high-flow riffle, mean between-day drift densities differed in 19/33 cases, 15 of which reflected increased drift following flow elevation on day 2.

Because the null hypothesis was not rejected in many instances, statistical power ($1 - \beta$) was calculated to assess the likelihood of a Type II error (i.e. not rejecting H_0 when it is false) (see Peterman 1990). Power was calculated following the procedures of Steel and Torrie (1980, p. 113 ff.) by expressing the observed effect size (i.e. mean between-day difference) in units of standard deviations (calculated from the error mean square term in the AOV). Theoretically, each experiment could correctly reject H_0 with 75% probability ($\beta = 0.25$) at $\alpha = 0.10$ if the observed effect was 0.67 standard deviations in size. Power for individual tests that did not reject H_0 was generally low, as shown in Table 2. In the reference riffle, low power resulted primarily from small effect size, while in the manipulated riffles, greater sampling variability tended to reduce statistical power. For example, in the low-flow riffle, H_0 was not rejected in many cases where between-day differences were relatively large (e.g. *Ephemerella infrequens*, *Hydropsyche* sp., and Chironomidae larvae in autumn; *Paraleptophlebia heteronea* and *Hydropsyche* sp. in spring). In these cases, large differences, coupled with low power, indicate low confidence in "accepting" H_0 (see Peterman 1990).

TABLE 1. Physical characteristics of three experimental riffles (reference, low flow, and high flow) before (day 1) and after (day 2) streamflow manipulations in each of three seasons.

	Reference			Low flow			High flow		
	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring
Wetted width (m)	5.6	5.6	5.6	6.1	6.1	6.1	5.0	5.0	5.0
Mean depth (cm)									
Day 1	35.8	33.3	38.7	30.2	23.5	35.3	37.3	31.0	49.0
Day 2	35.8	33.3	38.7	28.7	23.2	32.0	40.5	37.5	52.8
Mean velocity at nets ($cm \cdot s^{-1}$)									
Day 1	36.8	42.9	49.3	33.0	10.1	39.8	49.1	35.4	35.8
Day 2	36.8	42.9	49.3	19.6	9.4	18.8	62.2	62.8	57.4
Mean discharge through nets ($m^3 \cdot h^{-1}$)									
Day 1	184	202	247	165	39	200	246	171	179
Day 2	184	202	247	92	34	88	312	322	277

Among-riffle comparisons for each of the four sampling times are summarized statistically in Table 3 and are shown graphically in Fig. 1. Following *flow reduction*, several taxa responded strongly in at least one season, with both immediate, diurnal increases (*Baetis* spp., *Epeorus longimanus*, *Triznaka signata*, Simuliidae) and delayed, nocturnal increases (*Baetis* spp., *E. longimanus*, *P. heteronea*, *T. signata*, *Isoperla* sp., *Cultus aestivalis*, Simuliidae) in drift density. Only a few taxa showed significant ($p < 0.1$) decreases in drift density under low-flow conditions, and these responses were generally inconsistent across seasons (*P. heteronea*, *E. infrequens*, *Brachycentrus americanus*, *Lepidostoma ormea*, *T. signata*). Several taxa showed consistent across-season responses (e.g. *Baetis* spp., Simuliidae), but a few taxa varied in time and direction of drift response to decreased flow (e.g. *P. heteronea*, *T. signata*).

In response to *flow elevation*, taxa showed occasional diurnal increases (e.g. *E. infrequens*, *B. americanus*, *L. ormea*, *Hydropsyche* sp., Simuliidae) or nocturnal increases (e.g. *Baetis* spp. *E. longimanus*, *P. heteronea*, Simuliidae) in drift relative to the reference riffle. Occasional decreases in drift density under elevated flow were observed for some taxa (*T. signata*, *E. infrequens*, *Isoperla* sp., Chironomidae larvae).

Changes in Daily Drift Rate

Between-day differences in total daily drift numbers (i.e. unadjusted for flow rate) showed relative seasonal consistency for many taxa (Fig. 2). In the reference riffle, between-day differences were generally small, with two exceptions. First, *T. signata* showed large variability in autumn and spring. Second, drift rates for *Baetis* spp. and *P. heteronea* increased on day 2 during summer. This difference probably reflected the depressant effect of the relatively greater moonlight that occurred on day 1 (cf. Anderson 1966). Two general patterns of response were observed following *flow reduction*. One group of taxa exhibited an increased drift rate (*Baetis* spp., *E. longimanus*, Simuliidae, *B. americanus*) whereas a second group showed reduced drift rate or no change in drift rate (*P. heteronea*, *E. infrequens*, *T. signata*, *L. ormea*). After *flow elevation*, total daily drift rate increased markedly (relative to the reference riffle) for almost all taxa (Fig. 2).

Changes in Diel Drift Periodicity

Under unmanipulated flow conditions, most taxa exhibited a propensity toward nocturnal drift (Fig. 3). Patterns ranged from seasonally consistent and strongly nocturnal (*Baetis* spp., *P. heteronea*, *E. longimanus*, *T. signata*) or weakly nocturnal (*B. occidentalis*, *L. ormea*) to seasonally variable but generally nocturnal (*E. infrequens*, Simuliidae). Only larval Chironomidae (not shown) were consistently aperiodic, with from 40 to 60% of total daily drift occurring at night.

Following flow manipulation, changes in diel pattern (relative to between-day differences in the reference riffle) were observed for some taxa in some seasons, but patterns were variable. After *flow reduction*, a relative decline in nocturnal drift occurred in autumn (*Baetis* spp., *P. heteronea*, *E. infrequens*, *B. occidentalis*, *L. ormea*) and in spring (*Baetis* spp., *P. heteronea*, Simuliidae). An increase in nocturnal drift following reduced flow was observed only for Simuliidae (summer) and *L. ormea* (spring) (Fig. 3).

Diel alterations following *flow elevation* were less pronounced than those following flow reduction. They ranged from a slight decrease in nocturnal drift in autumn (*P. heteronea*, *E. infrequens*, *T. signata*, *B. occidentalis*, *L. ormea*) to a slight increase in nocturnal drift in summer (*E. infrequens*, Simuliidae) (Fig. 3). Larval Chironomidae showed a pronounced increase in diurnal drift density under high flow only in autumn, with nocturnal drift proportion dropping from 57 to 11%.

Discussion

The experimental flow manipulations in this study were comparable with the kind of hydrologic variation characteristic of the regulated upper Colorado River. Whole-channel discharge in the upper Colorado River is highly regulated by seasonally predictable, scheduled reservoir releases. Flow is maintained at only ~0.56 $m^3 \cdot s^{-1}$ from September through April, at which time it is roughly quadrupled from May through July before being halved for the month of August and again at the beginning of September. The changes in velocity, depth, and flow between these prescheduled "seasonal" discharge levels are comparable with those imposed by the experimental flow manipulations described above (e.g. cf. the unmanipulated depth and velocity values across seasons in each riffle with the between-day experimental differences in Table 1).

Experimental increases and decreases in streamflow during daylight hours each affected the magnitude and direction of change in drift density for several taxa from the same local

TABLE 2. Mean daily drift densities ((no. individuals·m^{-3})$^{0.5}$ × 1000) for combined three nets × four sampling times on day 1 (no manipulation) and day 2 (flow manipulation) for each of 14 taxa in three riffles (reference, low flow, and high flow) for each of three seasons. For each taxon, total day 1 and day 2 densities are to the left and right of the slash, respectively. Significant between-day increases within a riffle ($F_{1,6}$) are indicated for $p < 0.05$ (+), $p < 0.01$ (++), and $p < 0.001$ (+++). Decreases are indicated for $p < 0.05$ (−), $p < 0.01$ (−−), and $p < 0.001$ (−−−). Symbols in parentheses are for $p < 0.10$. For cases where the null hypothesis is not rejected, the statistical power ($1-\beta$) of the experiment to detect the observed between-day difference at $\alpha = 0.10$ is indicated by the letters a (25% < power ≤ 50%) and b (power ≤ 25%).

	Summer			Autumn			Spring		
Taxon	Reference	Low flow	High flow	Reference	Low flow	High flow	Reference	Low flow	High flow
Ephemeroptera									
Baetis spp.	1163/1267 +	1092/1747 +++	1085/1449 +++	352/348 b	529/844 ++	417/381 b	670/659 b	644/1216 +++	667/768 (+)
Paraleptophlebia heteronea	561/569 b	317/409 (+)	502/563 (+)	289/273 b	722/440 −	521/479 a	147/123 −	116/76 b	109/208 +++
Ephemerella infrequens	245/261 b	241/253 b	214/233 b	359/312 b	883/752 a	605/568 b	323/329 b	333/251 −	296/332 (+)
Epeorus longimanus	0/0	0/0	0/0	0/0	0/0	0/0	156/177 b	150/621 +++	189/275 +++
Plecoptera									
Triznaka signata	89/100 b	68/116 +	72/85 b	269/251 b	565/516 b	369/319 (−)	285/326 (+)	334/514 +++	302/291 b
Isoperla sp.	0/0	0/0	0/0	58/87 ++	163/213 b	126/142 b	109/102 b	98/131 (+)	73/104 a
Cultus aestivalis	0/0	0/0	0/0	0/0	0/0	0/0	38/32 b	29/72 +	19/40 (+)
Trichoptera									
Brachycentrus americanus	0/0	0/0	0/0	43/52 b	78/38 (−)	61/46 b	34/49 a	23/230 +++	47/110 +++
Lepidostoma ormea	0/0	0/0	0/0	68/111 b	140/198 b	92/136 (+)	179/161 b	101/0 −−	74/180 +++
Hydropsyche spp.	0/0	0/0	0/0	11/19 b	59/133 a	71/104 (+)	24/18 b	11/62 a	23/66 +
Glossosoma verdona	0/0	0/0	0/0	431/364 a	730/830 b	632/475 −	187/151 b	188/193 b	175/168 b
Diptera									
Simuliidae	233/214 b	206/540 +++	172/245 ++	152/153 b	372/1190 ++	209/288 +	252/256 b	331/636 ++	274/373 ++
Chironomidae larvae	360/333 b	344/444 +	328/328 b	56/78 a	169/231 b	146/37 −−−	166/147 b	181/267 (+)	186/187 b
Chironomidae pupae	474/454 b	440/410 b	354/393 a	11/14 b	10/0 b	12/5 b	163/132 b	146/158 b	158/132 (−)

populations occupying adjacent riffles. Several taxa responded to daytime flow changes with an immediate change in drift density, but typical nocturnal peaks were also generally enhanced under manipulated flow conditions, resulting in unmodified diel drift patterns. Seasonal differences both in magnitude and direction of altered drift densities and in propensity to exhibit modified diel pattern were observed. Changes in daily drift rate following flow manipulation showed that almost all taxa consistently exhibited increased drift rate under elevated flow. Under reduced flow, various taxa tended to show increased drift rate or to show decreased drift rate (or no change).

Many taxa appeared to respond more strongly to reduced flow than to elevated flow in terms of drift density (Fig. 1), but not necessarily in terms of drift rate (Fig. 2). The interpretation of drift patterns is strongly influenced by assumptions about the relationship between drift distance and water velocity. Drift density is most appropriate where distance varies linearly with velocity, which may not always be the case (see Elliott 1971; Allan and Feifarek 1989). Drift rate, by contrast, does not account for velocity-dependent drift distance. Interpretation can also be influenced by size of the available source of drifting organisms. In the low-flow riffle, the diversion structure prevented organisms from drifting into the riffle from upstream on day 2 (but not day 1) whereas in the high-flow riffle, organisms from undisturbed, upstream sources could pass through the riffle on both days. Despite these problems, a differential drift response to reduced versus elevated flow for several taxa was suggested by the observation that immediate (diurnal) responses

TABLE 3. Time-dependent responses of drift density to decreased/increased flow conditions for each of three seasons. Sample times are D1 (immediately following flow manipulation, 6 h before sunset), D2 (3 h before sunset), N1 (after sunset), N2 (3 h following sunset), and N3 (predawn). Between-day increase (+), decrease (−), and no change (0) relative to the between-day change in the reference riffle is to the left of the slash for the low-flow treatment and to the right for the high-flow treatment. Changes are significant at $p < 0.05$ unless in parentheses; then at $p < 0.1$ (Student–Newman–Keuls mltiple comparison).

	Summer				Autumn				Spring			
Taxon	D 2	N 1	N 2	N 3	D 1	D 2	N 1	N 2	D 1	D 2	N 1	N 2
Ephemeroptera												
Baetis spp.	+/0	+/+	+/0	0/0	0/0	+/0	(+)/0	(+)/0	+/0	+/0	+/0	(+)/0
Paraleptophlebia heteronea	0/0	+/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/+	−/+
Ephemerella infrequens	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/−	0/0	0/+	−/0	0/0
Epeorus longimanus	−	−	−	−	−	−	−	−	+/0	0/0	+/(+)	+/0
Plecoptera												
Triznaka signata	0/0	+/+	0/0	−/−	0/0	(−)/0	0/0	0/0	+/0	0/0	+/(−)	+/0
Isoperla sp.	−	−	−	−	0/0	0/0	0/0	0/−	0/0	0/0	0/0	+/(+)
Cultus aestivalis	−	−	−	−	−	−	−	−	0/0	0/0	+/0	0/(+)
Trichoptera												
Brachycentrus americanus	−	−	−	−	0/0	0/0	(−)/0	0/0	0/0	0/(+)	+/0	+/0
Lepidostoma ormea	−	−	−	−	0/0	0/0	0/0	0/0	0/(+)	0/(+)	(−)/0	0/0
Hydropsyche sp.	−	−	−	−	0/0	0/+	0/0	0/0	0/0	0/0	0/0	0/0
Glossosoma verdona	−	−	−	−	0/0	0/0	0/0	0/0	0/0	0/0	(+)/0	0/0
Diptera												
Simuliidae	+/0	+/(+)	0/0	0/0	(+)/0	0/0	(+)/0	+/0	+/(+)	(+)/+	+/0	0/0
Chironomidae larvae	0/0	0/(+)	0/0	0/0	0/0	0/−	0/−	0/−	0/0	0/0	0/0	0/0
Chironomidae pupae	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

to reduced flow were typically more pronounced than were responses to elevated flow. This argument assumes that diurnal drift following flow manipulation was uninfluenced by nonriffle source areas. This is not unreasonable given that organisms typically drifted in low numbers under unaltered flow conditions during daylight hours and that daytime drift distances for organisms departing the substrata can be short (Statzner and Mogel 1985).

As flow changes moderately, important correlates of habitat quality also change at the spatial scale relevant to benthic invertebrates. Adjustments in channel depth and current velocity can alter near-bed current velocity (Davis and Barmuta 1989), suspended sediment load, physiological exchange rates, rate at which suspended food particles are delivered, light penetration (Pearson and Franklin 1968), and amount of exposed shoreline (Hinckley and Kennedy 1972; Perry and Perry 1986). Many of the above factors probably did not contribute to the observed drift responses. For example, dissolved oxygen remained saturated at all times during the study and no appreciable change in seston was observed in the experimental riffles following flow manipulation (N.L. Poff, unpubl. data). Shoreline dewatering in the low-flow riffle did not occur during summer and spring; however, under conditions of low channel flow in autumn, about 10% of the areal surface of the riffle (visual estimate) was partially exposed as boulder surfaces protruded from the water following experimental flow reduction, and this could have contributed to elevated drift densities.

Perhaps most importantly, hydraulic characteristics of experimental riffles would have been modified due to changes in channel depth and current velocity following flow manipulation. Mean shear stress (an expression of erosive force) on the stream bottom can be expressed as varying directly with mean channel velocity and indirectly with the logarithm of depth divided by characteristic substratum size (see Statzner et al. 1988 for detailed methodology). In this study, substratum size within a riffle remained constant during each 2-d period. Therefore, because between-day changes in velocity due to manipulation were much greater than were logarithmic changes in depth (see Table 1), it can be inferred that the *average* erosive forces declined in the low-flow riffle and increased in the high-flow riffle in response to each seasonal flow manipulation. Changes in shear stress would be expected to directly affect probability of passive erosion of organisms from exposed surfaces (see Statzner et al. 1988). In addition to mean shear stress, a change in flow probably also influenced the extent to which turbulent eddies intruded into the spaces between the abundant, irregularly spaced boulders characteristic of the stream bottom. Turbulence is rarely measured, but it may be an important control on drift by increasing local shear stress (Statzner et al. 1984; Wilzbach et al. 1988; Wilzbach 1990) and by promoting prolonged suspension in the water column. We suspect that turbulence intensity varied directly with direction of flow change in the experimental riffles. Therefore, experimental changes in channel velocity would influence the erosion and transport components of drift, which, if an entirely passive process, would be expected to differ accordingly between the low-flow and high-flow riffles.

Whether drift is an active or passive phenomenon remains contentious (e.g. Allan et al. 1986; Wilzbach et al. 1988; Allan and Feifarek 1989; Wilzbach 1990). Multiple factors can influence drift, ranging from environmental conditions to species-specific attributes such as behavior, morphology, and mode of locomotion (Elliott 1971; Corkum 1978; Wilzbach et al. 1988) or activity and exposure to current (e.g. Elliott 1968). Without detailed behavioral observations for different taxa collected in this study, the mechanism(s) of drift entry and transport cannot be known. However, given that much of the debate over passive versus active drift relies on the presumed role of displacement and transport by current, the differential drift responses of various taxa to directional flow manipulations can be put in this context.

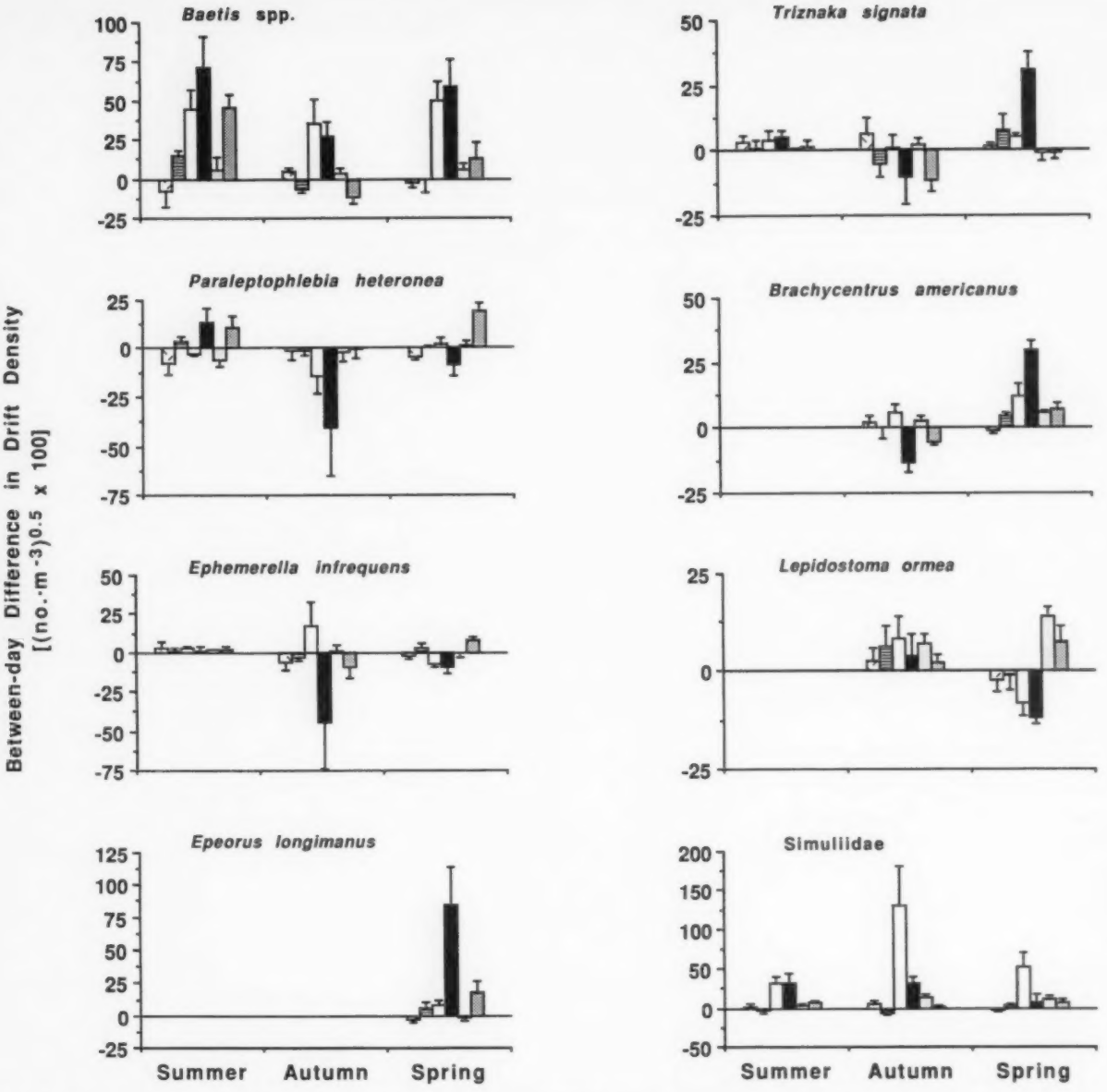

FIG. 1. Diurnal and nocturnal drift density responses of eight taxa to unaltered, experimentally decreased, and experimentally increased streamflow over three seasons. The ordinate represents the difference in drift density between day 2 (flow manipulation) and day 1 (no manipulation), each expressed as $(\text{no.}\cdot\text{m}^{-3})^{0.5} \times 100$. Mean between-day difference ± 1 SE are shown for diurnal samples in the reference (▨), nocturnal reference (▤), diurnal low-flow (□), nocturnal low-flow (■), diurnal high-flow (▩), and nocturnal high-flow (▦), riffle.

Active, behavioral abandonment of the substratum probably contributed to altered drift densities for some taxa, especially under reduced flows, where inferred mean shear stress on the bottom declined but drift increased. For example, simuliids, elsewhere reported to relocate immediately following flow reductions (e.g. Minshall and Winger 1968; Corrarino and Brusven 1983), are unlikely to be passively dislodged under moderate flow changes given their sophisticated hydrodynamic control (e.g. Chance and Craig 1986). Similarly, *Baetis* spp. showed some diurnal increase in drift following flow reduction, but they generally delayed their increased drift response until after sunset. Baetids are good swimmers and have often been reported to actively enter and remain in the drift under conditions of low velocity (e.g. Minshall and Winger 1968; Corkum et al. 1977; Corkum 1978; Ciborowski 1983; Allan and Feifarek 1989). The consistent seasonal responses to low flow observed in this study are consistent with active entry and transport. Interestingly, small baetids, presumably less susceptible to trout predation than are larger individuals (see Allan 1978), were significantly overrepresented in the diurnal drift following flow reduction during spring and summer (Poff et al. 1991). Another mayfly, *E. longimanus*, also exhibited some initial

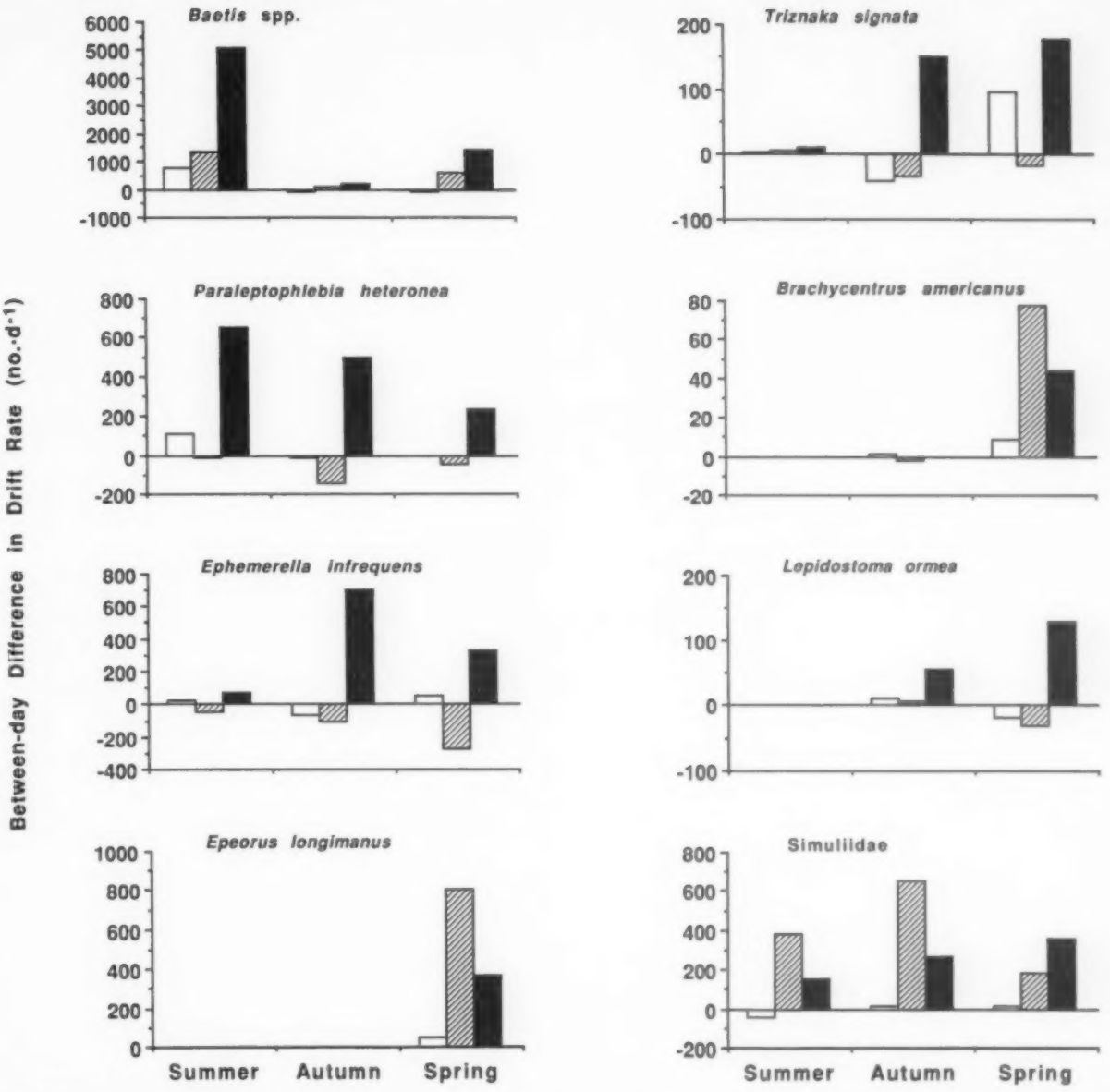

FIG. 2. Drift rate response of eight taxa to unaltered (□), experimentally decreased (▨), and experimentally increased (■) streamflow over three seasons. The ordinate represents the difference in total untransformed drift numbers between day 1 (no manipulation) and day 2 (flow manipulation).

diurnal increase in drift following flow reduction, but this increase was small relative to the very large nocturnal response (cf. Gore 1977; Hooper and Ottey 1988). This and other heptageneiid species are capable of rapid movement across the substratum surface (N. L. Poff, pers. obs.; see Allan et al. 1986) and are unlikely to be passively displaced as current declines. However, this species may actively drift under low current (see Allan and Feifarek 1989).

Under elevated flow, these three taxa responded with relatively small increases in drift density but with more pronounced increases in drift rate (Fig. 2). Passive dislodgement under experimentally increased shear cannot be ruled out, especially for *Baetis* spp., which consistently showed a greater drift rate in the high-flow versus low-flow riffle. Drift rates for *E. longimanus* and Simuliidae (except spring) were greater in the low-flow than in the high-flow riffle, which runs counter to the expectation of a purely passive displacement.

Some taxa appeared to be passive drifters, at least seasonally, as suggested by the coincident patterns of increased drift under elevated flow and decreased drift under reduced flow. The best example was provided by *L. ormea*, a mobile caddisfly with a horn-shaped sand case. This species occurs in relatively shel-

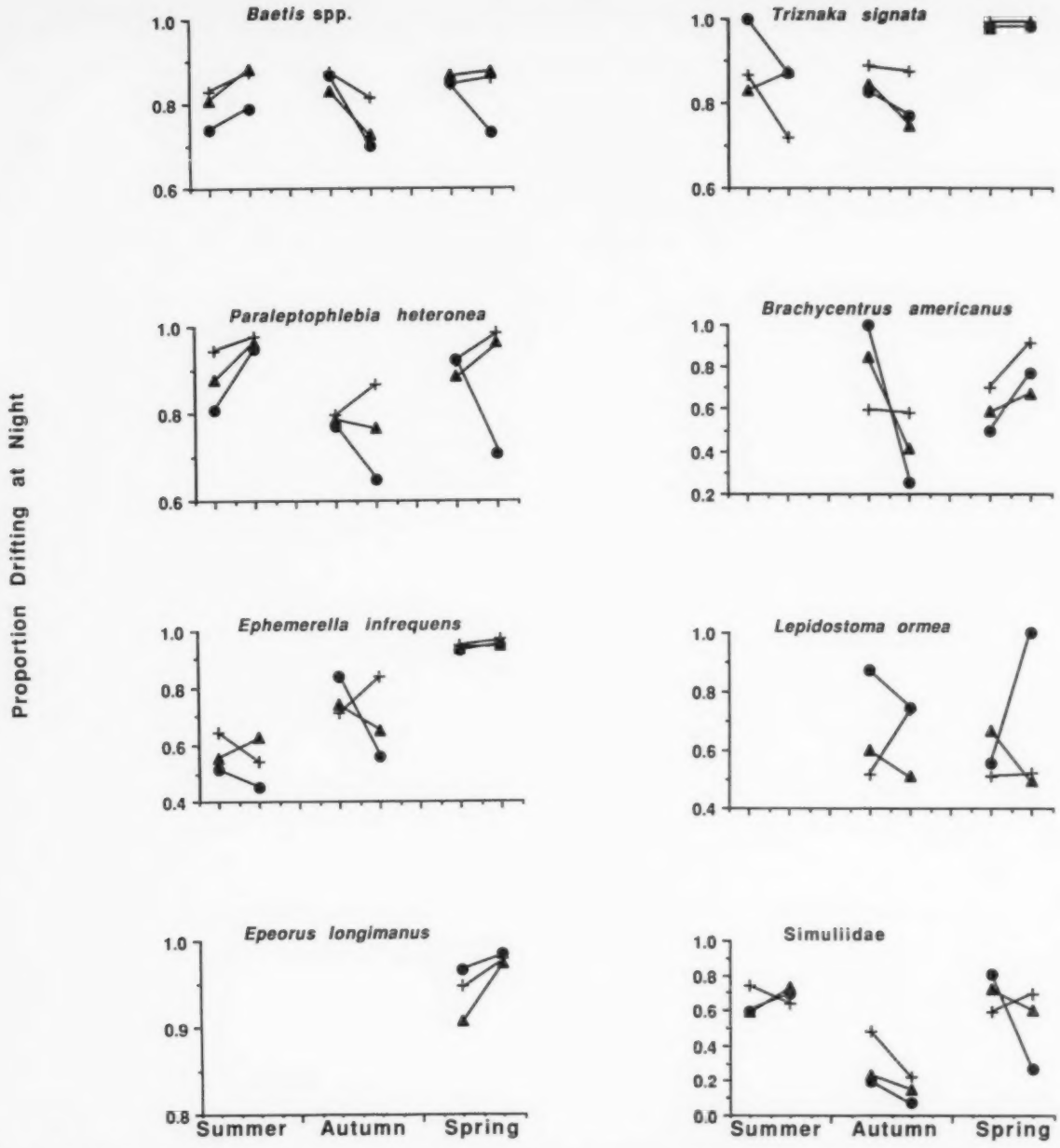

FIG. 3. Diel drift patterns of eight taxa under unaltered (+), experimentally decreased (●), and experimentally increased (▲) streamflow over three seasons. The proportion drifting at night is calculated as nocturnal density/(diurnal density + nocturnal density). Within each season, between-day differences in the proportion drifting at night are shown by the line connecting symbols on the left (day 1) with symbols on the right (day 2).

tered microhabitats in the regulated Colorado River (N. L. Poff, pers. obs.; Rader and Ward 1990) and was generally uncommon in the drift. Following flow elevation in the spring, drift density (Fig. 1) and drift rate (Fig. 2) increased markedly. However, following flow reduction, zero *L. ormea* were collected in the diurnal drift. These responses demonstrably altered the diel drift periodicity for this species (Fig. 3). Two mayfly species, *P. heteronea* and *E. infrequens*, also showed seasonally consistent, flow-dependent directional responses in drift rate that suggested a large passive component in their drift (see Fig. 2). The pattern of drift density was less supportive, although there was some seasonal decline in nocturnal drift density following flow reduction that occasionally enhanced relative diurnal drift.

For many taxa, seasonal variation in drift responses to flow manipulations further complicates interpretation. This seasonal

variation may reflect numerous factors, including ontogenetic changes in hydrodynamic morphology (Statzner 1988), microhabitat selection, and growth rates (Kohler 1983), all of which may affect responses to altered hydrologic conditions. Additionally, differences in background environmental conditions (e.g. level of whole-channel flow) and variable intensity of experimental manipulations may have influenced seasonal drift responses to flow alterations. Methodological problems (e.g. preferential weighting of nocturnal responses in summer when the first diurnal sample was lost) and low statistical power due to high sampling variability (see Table 2) also complicated cross-seasonal generalizations.

Irrespective of precise mechanisms regulating drift entry, and seasonal variation notwithstanding, flow-dependent changes in drift density and drift rate for many taxa were observed following experimental discharge manipulations. These numerous responses emphasize the importance of hydrologic regime in influencing drift patterns. Some evidence suggests that the magnitudes of invertebrate drift responses to flow fluctuations are directly related to how much time has passed since the last flow fluctuation (Irvine 1985; Perry and Perry 1986; Irvine and Scott 1988). Where hydrologic conditions remain stable over prolonged periods, organisms presumably have greater opportunities to affiliate themselves with specific microhabitat conditions. The temporally stable flow in the regulated Colorado River prior to these experiments suggests that the flow manipulations represented relatively pronounced hydrologic disruptions. The experimental flow manipulations were executed during the daylight hours in an attempt to induce shifts in the diel pattern of drift density for invertebrate taxa. However, for most numerically abundant species, flow-induced alterations in drift density were generally not accompanied by a shift in the diel periodicity of drift, suggesting that moderate hydrologic fluctuations to not substantially increase diurnal drift densities (although catastrophic fluctuations may, e.g. Minshall and Winger 1968). Many natural lotic systems are characterized by much greater temporal streamflow variability than the regulated Colorado River (see Poff and Ward 1989). In these hydrologically more variable systems, microhabitat conditions should be less stable over long periods of time, and even less dramatic drift responses of invertebrates to flow fluctuations of magnitudes similar to those induced in this study would be predicted.

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References

ALLAN, J. D. 1978. Trout predation and the size composition of stream drift. Limnol. Oceanogr. 23: 1231–1237.

ALLAN, J. D., AND B. P. FEIFAREK. 1989. Distances travelled by drifting mayfly nymphs: factors influencing return to the substrate. J. N. Am. Benthol. Soc. 8: 322–330.

ALLAN, J. D., A. S. FLECKER, AND N. L. MCCLINTOCK. 1986. Diel epibenthic activity of mayfly nymphs, and its nonconcordance with behavioral drift. Limnol. Oceanogr. 31: 1057–1065.

ALLAN, J. D., AND E. RUSSEK. 1985. The quantification of stream drift. Can. J. Fish. Aquat. Sci. 42: 210–215.

ANDERSON, N. H. 1966. Depressant effect of moonlight on activity of aquatic insects. Nature (Lond.) 209: 319–320.

ANDERSON, N. H., AND D. M. LEHMKUHL. 1968. Catastrophic drift of insects in a woodland stream. Ecology 49: 198–206.

BRITTAIN, J. E., AND T. J. EIKELAND. 1988. Invertebrate drift — a review. Hydrobiologia 166: 77–93.

BROOKER, M. P., AND R. J. HEMSWORTH. 1978. The effect of the release of an artificial discharge of water on invertebrate drift in the R. Wye, Wales. Hydrobiologia 59: 155–163.

CARPENTER, S. R. 1990. Large-scale perturbations: opportunities for innovation. Ecology 71: 2038–2043.

CARPENTER, S. R., J. F. KITCHELL, J. R. HODGSON, P. A. COCHRAN, J. J. ELSER, M. M. ELSER, D. M. LODGE, D. KRETCHMER, X. HE, AND C. N. VON ENDE. 1988. Regulation of lake primary productivity by food web structure. Ecology 68: 1863–1876.

CHANCE, M. M., AND D. A. CRAIG. 1986. Hydrodynamics and behavior of Simuliidae larvae (Diptera). Can. J. Zool. 64: 1295–1309.

CIBOROWSKI, J. J. H. 1983. Influence of current velocity, density and detritus on drift of two mayfly species. Can. J. Zool. 61: 119–125.

CORKUM, L. D. 1978. The influence of density and behavioral type on the active entry of two mayfly species (Ephemeroptera) into the water column. Can. J. Zool. 56: 1201–1206.

CORKUM, L. D., P. J. POINTING, AND J. J. H. CIBOROWSKI. 1977. The influence of current velocity and substrate on the distribution and drift of two species of mayflies (Ephemeroptera). Can. J. Zool. 55: 1970–1977.

CORRARINO, C. A., AND M. A. BRUSVEN. 1983. The effects of reduced stream discharge on insect drift and stranding of near shore insects. Freshwater Invertebr. Biol. 2: 88–98.

CRISP, D. T., AND S. ROBSON. 1979. Some effects of discharge upon the transport of animals and peat in a north Pennine headstream. J. Appl. Ecol. 16: 721–736.

DAVIS, J. A., AND L. A. BARMUTA. 1989. An ecologically useful classification of mean and near-bed flows in streams and rivers. Freshwater Biol. 21: 271–282.

ELLIOTT, J. M. 1968. The daily activity patterns of mayfly nymphs (Ephemeroptera). J. Zool. Lond. 155: 201–221.

1971. The distances travelled by drifting invertebrates in a Lake District stream. Oecologia 6: 191–220.

1973. The food of brown and rainbow trout (*S. trutta* and *S. gairdneri*) in relation to the abundance of drifting invertebrates in a mountain stream. Oecologia 12: 329–347.

GORE, J. A. 1977. Reservoir manipulations and benthic macroinvertebrates in a prairie river. Hydrobiologia 55: 113–123.

GRAESSER, A. K. 1988. Invertebrate drift in three flood-prone streams in South Westland, New Zealand. Verh. Int. Ver. Limnol. 23: 1422–1426.

HINCKLEY, T. M., AND H. D. KENNEDY. 1972. Fluctuations of aquatic and terrestrial invertebrates in drift samples from Convict Creek, California. Northwest Sci. 46: 270–276.

HOOPER, F. F., AND D. R. OTTEY. 1988. Responses of macroinvertebrates of two headwater streams to discharge fluctuations. Verh. Int. Ver. Limnol. 23: 1159–1166.

HURLBERT, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187–211.

IRVINE, J. R. 1985. Effects of successive flow perturbations on stream invertebrates. Can. J. Fish. Aquat. Sci. 42: 1922–1927.

IRVINE, J. R., AND P. R. HENRIQUES. 1984. A preliminary investigation on effects of fluctuating flows on invertebrates of the Hawea River, a large regulated river in New Zealand. N.Z. J. Mar. Freshwater Res. 18: 283–290.

IRVINE, J. R., AND D. SCOTT. 1988. Impacts on drifting invertebrates of stream flow changes occurring in the afternoon and morning. Verh. Int. Ver. Limnol. 23: 1432–1436.

KOHLER, S. L. 1983. Positioning on substrates, positioning changes, and diel drift periodicities in mayflies. Can. J. Zool. 61: 1362–1368.

MCLAY, C. L. 1968. A study of drift in the Kakanui River, New Zealand. Aust. J. Mar. Freshwater Res. 19: 139–149.

MINSHALL, G. W., AND F. V. WINGER. 1968. The effect of reduction in stream flow on invertebrate drift. Ecology 49: 580–582.

PEARSON, W. D., AND D. R. FRANKLIN. 1968. Some factors affecting drift rates of *Baetis* and Simuliidae in a large river. Ecology 49: 75–81.

PERRY, S. A., AND W. B. PERRY. 1986. Effects of experimental flow regulation on invertebrate drift and stranding in the Flathead and Kootenai rivers, Montana, USA. Hydrobiologia 134: 171–182.

PETERMAN, R. M. 1990. Statistical power analysis can improve fisheries research and management. Can. J. Fish. Aquat. Sci. 47: 2–15.

POFF, N. L., R. D. DECINO, AND J. V. WARD. 1991. Size-dependent drift responses of mayflies to experimental hydrologic variation: active predator avoidance or passive hydrodynamic displacement? Oecologia. (In press)

POFF, N. L., AND J. V. WARD. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. Can. J. Fish. Aquat. Sci. 46: 1805–1818.

RADER, R. B., AND J. V. WARD. 1988. Influence of regulation on environmental conditions and the macroinvertebrate community in the upper Colorado River. Regul. Rivers 2: 597–618.

1990. Diel migration and microhabitat distribution of a benthic stream assemblage. Can. J. Fish. Aquat. Sci. 47: 711–718.

RADFORD, D. S., AND R. HARTLAND-ROWE. 1971. A preliminary investigation of bottom fauna and invertebrate drift in an unregulated and a regulated stream in Alberta. J. Appl. Ecol. 8: 883–903.

SCULLION, J., AND A. SINTON. 1983. Effects of artificial freshets on substratum composition, benthic invertebrate fauna and invertebrate drift in two impounded rivers in mid-Wales. Hydrobiologia 107: 261–269.

STATZNER, B. 1988. Growth and Reynolds number of lotic macroinvertebrates: a problem for adaptation of shape to drag. Oikos 51: 84–87.

STATZNER, B., C. DEJOUX, AND J.-M. ELOUARD. 1984. Field experiments on the relationship between drift and benthic densities of aquatic insects in tropical streams (Ivory Coast). Rev. Hydrobiol. Trop. 17: 319–334.

STATZNER, B., J. A. GORE, AND V. H. RESH. 1988. Hydraulic stream ecology: observed patterns and potential applications. J. N. Am. Benthol. Soc. 7: 307–360.

STATZNER, B., AND R. MOGEL. 1985. An example showing that drift net catches of stream mayflies (*Baetis* spp., Ephemeroptera, Insecta) do not increase during periods of higher substrate surface densities of the larvae. Verh. Int. Ver. Limnol. 22: 3238–3243.

STEEL, R. G. D., AND J. H. TORRIE. 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. McGraw-Hill, New York, NY. 633 p.

STEWART-OATEN, A., W. W. MURDOCH, AND K. R. PARKER. 1986. Environmental impact assessment: "pseudoreplication" in time? Ecology 67: 929–940.

WATERS, T. F. 1962. Diurnal periodicity in the drift of stream invertebrates. Ecology 43: 316–320.

1972. The drift of stream insects. Annu. Rev. Entomol. 17: 253–272.

WILZBACH, M. A. 1990. Nonconcordance of drift and benthic activity in *Baetis*. Limnol. Oceanogr. 35: 945–952.

WILZBACH, M. A., K. W. CUMMINS, AND R. A. KNAPP. 1988. Toward a functional classification of stream invertebrate drift. Verh. Int. Ver. Limnol. 23: 1244–1254.

A Statistically Valid Model of the Morphoedaphic Index[1]

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Rempel, R. S., and P. J. Colby. 1991. A statistically valid model of the morphoedaphic index. Can. J. Fish. Aquat. Sci. 48: 1937–1943.

The morphoedaphic index (MEI) has been criticized because of the use of ratio variables in linear regression. Computationally simple, the continued use of the index is questionable given the widespread access fisheries biologists now have to computerized statistical packages. We present a statistically valid analogue to the MEI, the morphoedaphic model (MEM), that utilizes multiple regression to characterize the morphometric and fertility properties of lakes to predict annual fish yield. Surface area, lake volume, and total dissolved solids (TDS) are used to predict annual fish yield for the lake and to derive associated confidence limits. Predicted yield of the newly derived model was compared with predictions from the original MEI. Comparisons were also made based on models derived from Ontario sport and commercial fisheries data sets. The MEM derived from these partitioned data sets more accurately modelled the observed long-term yields for these lakes. Analysis of the remaining outliers suggests that several additional variables and stratification may be required to further develop the precision of the statistical model.

L'utilisation de l'indice morphoédaphique (IME) a fait l'objet de critiques à cause de l'application de variables de rapports à des régressions linéaires. Compte tenu du fait que des biologistes spécialistes des pêches ont facilement accès à des ensembles de programmes statistiques informatisés, et malgré la simplicité de la détermination de l'indice, il est permis de remettre en question l'utilisation de l'indice. Nous présentons un analogue statistiquement valable de l'IME, soit le modèle morphoédaphique (MEM); ce dernier fonctionne par régressions multiples afin de caractériser les propriétés morphométriques et de fertilité des lacs afin de prévoir le rendement annuel en poisson. La superficie et le volume des lacs ainsi que les matières totales dissoutes servent à calculer le rendement annuel prévu en poisson d'un lac ainsi qu'à calculer par dérivation les limites de confiance associées. Le rendement prévu par le nouveau modèle a été comparé aux prévisions de l'IME original. On a également fait des comparaisons à partir de modèles dérivés des ensembles de données sur la pêche sportive et la pêche commerciale en Ontario. Le MEM dérivé de ces ensembles distincts de données a modélisé les rendements à long terme des lacs avec plus de précision que l'IME. L'analyse des valeurs aberrantes restantes semble indiquer qu'il faudra inclure plusieurs variables additionnelles et procéder à des stratifications afin d'améliorer la précision du modèle statistique.

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The morphoedaphic index, or MEI (Ryder 1965), is a simple predictive tool used by fisheries managers to estimate annual fish harvest based on the mean depth, surface area, and total dissolved solids (TDS) of the lake. MEI is the ratio of TDS to mean depth, and Ryder (1965) derived the model

$$H = b\,(\mathrm{MEI})^{0.45}$$

where H is annual harvest of fish and b is a constant dependent on scale of measure.

The MEI equation has been criticized because of its use of derived ratio variables in development of the model (Jackson et al. 1990). For example, mean depth is often determined as a ratio of lake volume to surface area. Jackson et al. (1990) concluded that the use of such variables results in unknown distribution of the errors associated with the model. Hence, valid estimates of error and confidence are not possible for dependent variables or model parameters. Others have criticized ratios in general because they obscure insights into empirical relationships among variables and hinder interpretation of data (Green 1979).

Historically, the model was developed as a tool for managers prior to the widespread access of digital computers (or even hand-held calculators) and associated statistical software. This was in part the reason for developing a simple tool for managers that required no complex computation (Ryder 1965, 1982). Access to statistical software is no longer an issue, and the continued use of a statistically weak model is now difficult to justify. Hence, we propose here a simple linear model of the MEI, the morphoedaphic model (MEM), that is defensible statistically.

Our purpose here is not to argue the validity of data transformations for weighted least squares of the type $Y' = Y/X$ and $X' = 1/X$, although some statisticians propose such a dual transformation when the variance of the regression residuals

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increases with Y (Chatterjee and Price 1977), and others argue that there is confusion over the statistical basis of the problem (Prairie and Bird 1989). Rather, the purpose is to explain the underlying concepts in the MEM, to describe development of the statistical model based on the more widely accepted logarithmic transformation, and to compare and contrast model predictions with the original MEI predictions. This statistical framework is then applied to a fisheries management problem to illustrate how differences in data sets affect the estimate of model parameters.

Model Development and Evaluation

Underlying Concepts of the MEM: Strengths and Weaknesses

The model uses three variables (surface area, lake volume, and TDS) to capture two limnological properties related to lake productivity: lake thermodynamics and lake fertility. Verbally, the model states that given lakes of equal area, cold and infertile lakes tend to produce fewer fish than warm and fertile lakes.

Two variables (surface area and lake volume) are required to describe lake thermodynamics because heat retention depends on allometric properties of basin morphometry. Although most lakes increase in volume in a constant manner with surface area, some lakes are outliers to the relationship, i.e. small (cold), deep lakes, such as the kettle basins. On the other side of the line lie large (warm), shallow lakes. Hence the need for two variables to characterize thermal retention. However, the dynamics of heat within the lake are complex. The partitioning and mixing of heat and water volume between the epilimnion and hypolimnion (Christie and Regier 1988), timing of thermal stratification and subsequent turnover, number of growing degree-days above a relevant physiological threshold (Colby and Nepszy 1981), diel and seasonal thermal variability, and mean and median seasonal temperatures (Schlesinger and Regier 1982) may all influence aquatic production through such mechanisms as growth, development rate, and age at first reproduction (e.g. Rempel and Carter 1987; Regier et al. 1990). Some of these thermal factors, such as thermal mixing and average temperature, are at least partially accounted for by the two morphometric variables: narrow, deep lakes in the north are generally colder than wide, shallow lakes because the water column mixes less and a greater proportion of the lake volume is colder hypolimnion. However, thermal dynamics are not fully accounted for by the morphometric variables, and this affects both the generality and precision of the statistical model. For example, growing degree-days, average temperature, seasonal variability, and thermal stratification will differ between higher and lower latitudes, independent of morphometry. The lake's source of water, e.g. surface runoff versus glacial melt, will affect average temperature, independent of lake morphometry within a given range of latitudes.

Increased model precision can be achieved by restricting or partitioning the data set to lakes with similar environmental conditions and latitude, or increased generality could be achieved by broadening the range of lakes in the model data set and then including additional variables, such as latitude, average epilimnion depth, average lake temperature (weighted for epi- and hypolimnion depth), etc., to account for the increased variability in thermal parameters. The decision to strive for generality or precision is based on cost, the needs of the manager, and the scientific objective.

The second property, fertility, is partially (although not entirely) independent of basin morphometry. It can be affected by watershed geomorphology, atmospheric depositions, point-source inputs, water exchange rates, etc. Under most circumstances, it has little influence beyond that described by basin morphometry. In special circumstances, it has a very strong additional influence. In the MEM, TDS is used to indicate fertility, but other correlates such as phosphorus (Downing et al. 1990), chlorophyll *a*, or primary and secondary productivity (Oglesby 1977, 1982; Oglesby et al. 1987; Leach et al. 1987) might better characterize fertility and could also be appropriate. If nutrients are being consumed rapidly, then indices of nutrient status at a single point in time, such as TDS or chlorophyll *a*, may insufficiently characterize fertility. Estimates of nutrient dynamics or nutrient loading may thus be useful additions to characterize lake fertility, but once again, the decision to increase precision and generality is based on costs, benefits, and objectives. For example, if the scientific objective is to predict fish production anywhere in the world, then additional thermal and fertility parameters will be necessary to account for conditions that differ from Ryder's (1965) predominantly oligotrophic lakes. Without such additional variables, the relationship between fish production and morphoedaphic variables is likely to be weak (e.g. Downing et al. 1990). Such an objective, however, is seldom required by fisheries managers. For them, greater precision, not generality, is often desired.

Data Conversions

Data for the first part of this paper were taken from Ryder (1965) and converted to SI units. Ryder presented mean depth and surface area as morphometric variables, and we calculated volume (cubic metres) from the relationship mean depth (metres) = volume (cubic metres)·surface area (hectares)$^{-1}$·10^4. We calculated annual harvest from the relationship yield = annual harvest·area^{-1} where yield is kilograms per hectare per year, annual harvest is kilograms per year, and area is hectares.

Log-linear MEM Model

To illustrate the above concepts, consider a first-order linear model of Ryder's (1965) data that relates lake area to fish harvest. Considering no other factors, fish harvest can be expected to increase allometricly with lake surface area because a given proportional (i.e. geometric) increase in surface area results in a different proportional increase in harvest. This relationship can be described mathematically by

$$\text{Harvest} = k\ \text{area}^b$$

where k is a constant and the parameter b does not equal 1.

In log-linear form, Ryder's (1965) data are described by

$$\log_e(\text{harvest}) = 0.840 \log_e(\text{area}) + 3.041$$

where harvest is annual mass (kilograms) of fish caught and kept in the lake per year and area is hectares.

It is clear that this trivial relationship describes (as expected) most of the variation (approximately 95%) in fish harvest among lakes (Tables 1 and 2). With the addition of lake volume, the effects of lake morphometry are better accounted for by

$$\log_e(\text{harvest}) = 1.586 \log_e(\text{area}) - 0.564 \log_e(\text{volume}) + 7.666$$

TABLE 1. MEM regression coefficients for Ryder's (1965) data (see Table 3 for names of the lakes). Partial coefficients estimate percentage of variability accounted for by the included variable, with the effects of the other variables held constant. Model coefficient represents the cumulative proportion of variability accounted for by the three variables, with the significance of the individual variables.

Variable entered	Partial r^2	Model R^2	Prob > F
$\log_e$ area	0.9479	0.9479	0.0001
$\log_e$ volume	0.0253	0.9732	0.0003
$\log_e$ TDS	0.0064	0.9796	0.0243

TABLE 2. MEM parameter estimates for Ryder's (1965) data. Intercept and slope values, and associated standard errors, for multiple regression. Prob > $|t|$ represents probability parameter is not significantly different from zero. In all cases, null hypothesis of no effect is rejected at alpha = 0.05.

Variable	Parameter estimate	Standard error	Prob > $\|t\|$
Intercept	6.184	1.181	0.0001
$\log_e$ area	1.589	0.156	0.0001
$\log_e$ volume	−0.561	0.116	0.0001
$\log_e$ TDS	0.294	0.120	0.0243

where volume is cubic metres.

This result indicates that basin morphometry accounts for 97% of variation in fish harvest. The unexplained variation is partially due to lakes with unusually high or low TDS. By including TDS, harvest is predicted by

$$(1)\quad \log_e(\text{harvest}) = 1.588 \log_e(\text{area}) - 0.561 \log_e(\text{volume}) + 0.293 \log_e(\text{TDS}) + 6.184$$

where TDS is milligrams per litre. This factor accounts for an additional 0.64% of variability in harvest per lake (Tables 1 and 2).

The interpretation of regression coefficients can be deceptive. For example, Youngs and Heimbuch (1982) suggested dropping TDS from the MEI because it only accounted for an additional 1% of the variability. For most lakes, surface area adequately predicts annual harvest, and it would be tempting to drop the latter two variables (Table 3). For lakes with "atypical" basin morphometry (e.g. Erie, Lake of the Woods, St. Clair lakes) or TDS levels (e.g. Reindeer, Wollaston, Heming lakes), predictions using surface area alone result in a substantial difference from the observed harvest.

By atypical, we simply mean atypical with respect to the original MEI data set. These lakes would be "outliers" for a regression with just lake area as the predictive variable, but are no longer outliers with all three variables in the model. Thus, volume and TDS essentially broaden the range of limnological conditions for which the model can validly be applied, but add little to the predictive accuracy of "typical" lakes.

Nonlinear MEM Model

The lack of heteroscedacity in the covariance matrix ($p < 0.0001$) indicates that the coefficients of the MEM are linearly additive and that the model should be equivalent in both linear and nonlinear forms. In support of this, Bajdik and Schneider (1991) concluded that the log-normal (and gamma) distributions were appropriate models of the error term for regressions of MEI linear models on fish yield. This was confirmed by

TABLE 3. Observed, MEI-predicted, and MEM-predicted annual fish yields. MEM-predicted yields are provided for three-variable (3v), two-variable (2v), and one-variable (1v) models. Values are $kg \cdot ha^{-1} \cdot yr^{-1}$.

Lake	Observed harvest	MEI	MEM (3v)	MEM (2v)	MEM (1v)
Superior	0.93	0.93	0.87	1.00	1.64
Huron	1.55	1.90	1.75	1.67	1.72
Michigan	1.78	1.63	1.44	1.37	1.73
Great Slave	1.61	2.08	1.80	1.60	1.96
Erie	8.18	4.13	3.93	3.24	1.97
Ontario	1.11	1.88	1.56	1.37	2.06
Athabaska	1.68	2.01	2.15	2.55	2.38
Reindeer	2.24	2.03	2.39	3.20	2.52
Nipigon	1.20	1.83	1.63	1.65	2.58
Lake of the Woods	5.15	4.03	4.53	4.88	2.71
Wollaston	2.24	1.77	2.02	2.80	2.95
St. Clair	6.41	9.36	9.85	8.17	3.23
La Ronge	3.36	4.22	3.99	3.63	3.23
Cree	2.24	1.83	2.22	3.33	3.24
Rainy	4.44	3.17	3.77	4.84	3.38
Big Peter Pond	7.39	3.96	3.68	3.44	3.65
Île à la Crosse	3.36	5.68	5.31	4.56	3.77
Churchill	3.36	4.72	4.57	4.30	3.79
Little Peter Pond	6.72	6.25	6.24	5.81	4.33
Waskesiu	7.84	5.03	4.31	3.73	5.08
Mountain	3.92	5.36	4.40	3.59	5.29
Otter	3.92	4.60	4.10	3.82	5.30
Heming	5.60	5.52	5.97	7.34	8.60

estimating parameters of the MEM using nonlinear regression techniques.

The nonlinear form of the model is stated as

$$(2)\quad \log_e(\text{harvest}) = \log_e(\text{area}^{b1} \cdot \text{vol}^{b2} \cdot \text{TDS}^{b3} \cdot \text{EXP}^{b0})$$

where parameters $b1$, $b2$, and $b3$ are equivalent to the slopes of the log-linear model and $b0$ is equivalent to the intercept.

Regression analysis was done using both the Gausian method (with partial derivatives declared) and the secant method (SAS Institute Inc. 1988). Estimates of regression parameters and standard errors were identical to those obtained using the log-linear multiple regression technique. Expression (2) without logarithms gives

$$(3)\quad \text{Harvest} = \text{area}^{b1} \cdot \text{vol}^{b2} \cdot \text{TDS}^{b3} \cdot \text{EXP}^{b0}.$$

In metric units, this yields

$$\text{Harvest} = \text{area}^{1.59} \cdot \text{vol}^{-0.56} \cdot \text{TDS}^{0.29} \cdot \text{EXP}^{6.18}.$$

In imperial units:

$$\text{Harvest} = \text{area}^{1.59} \cdot \text{vol}^{-0.56} \cdot \text{TDS}^{0.29} \cdot \text{EXP}^{7.53}$$

where harvest is now annual weight in pounds, area in acres, volume is cubic feet, and TDS is parts per million.

Note that only the "intercept," $b0$, changes with the imperial equation. The functional relationships described by $b1$, $b2$, and $b3$ are independent of scale of measure, although error estimates will change with scale of measure (Bajdik and Schneider 1991).

Comparison of MEI and MEM

Comparison of the MEI and MEM results indicates that the two methods are very close in their predictions of fish yield for

TABLE 4. MEM-predicted annual harvest (millions of kg) and asymmetrical 95% confidence limits. Confidence limits include variability of model error term and parameter estimates.

Lake	Predicted	Lower limit	Upper limit
Superior	7.16	3.15	16.27
Huron	10.44	4.78	22.77
Michigan	8.38	3.81	18.40
Great Slave	4.89	2.24	10.67
Erie	10.11	4.48	22.78
Ontario	3.03	1.37	6.70
Athabaska	1.70	0.79	3.65
Reindeer	1.33	0.60	2.94
Nipigon	0.79	0.36	1.72
Lake of the Woods	1.62	0.73	3.57
La Ronge	0.47	0.22	1.00
Cree	0.26	0.11	0.58
Rainy	0.34	0.15	0.74
Big Peter Pond	0.20	0.095	0.43
Île à la Crosse	0.24	0.11	0.51
Churchill	0.20	0.093	0.42
Little Peter Pond	0.12	0.054	0.26
Waskesiu	0.030	0.014	0.067
Mountain	0.024	0.010	0.053
Otter	0.022	0.0099	0.048
Heming	0.0015	0.00065	0.0037

the original data set (Table 3). The functional relationship between the two forms of the model can be better explored by casting the MEI into a full exponential form. First:

$$\text{Yield} = k(T \cdot D^{-1})^{0.45}$$

where yield = harvest (C) divided by area (A), T is total dissolved solids, D = volume (V) divided by A, and k is a constant. Next, expressing the MEI in an exponential form yields

$$CA^{-1} = k(T^1((V^1 \cdot A^{-1})^{-1}))^{0.45}$$
$$CA^{-1} = k(A^1 \cdot V^{-1} \cdot T^1)^{0.45}$$
$$CA^{-1} = k(A^{0.45} \cdot V^{-0.45} \cdot T^{0.45})$$
$$C = k(A^{1.45} \cdot V^{-0.45} \cdot T^{0.45}) \quad (4)$$

where $V = D \times A \times 10^4$ (in metric units)
and thus, $k = \text{EXP}^{(4.48113)} = 88.3344$.

Expressing the MEM (equation (3)) in exponential form yields

$$C = i(A^{1.59} \cdot V^{-0.56} \cdot T^{0.29}) \quad (5)$$

where $i = \text{EXP}^{b0}$ of equation (3) and $b0 = 6.18$.

In this form, it is clear that the MEI (4) is closely related to the MEM (5). In (4), the model term $(A^{1.45} \cdot V^{-0.45} \cdot T^{0.45})$ represents the MEI, and TDS is assigned greater importance in this model than in (5). Also, this form (5) is similar to that derived by Schneider and Haedrich (1989), but we used lake volume and surface area, rather than mean depth and surface area, as the morphometric variables.

It is clear from this that regardless of the statistical "weakness" of the MEI approach, the MEI-predicted harvest differs little from the statistically valid MEM approach, and the problem of "spurious correlation" (Jackson et al. 1990) is not evident in the predicted harvest. The MEM, however, allows for valid estimates of statistical error, such as 95% confidence limits (Table 4). These confidence limits indicate the variability not accounted for by the regression and the uncertainty (error) in estimation of the regression parameters.

TABLE 5. Partial and simple Pearson correlation coefficients of MEM regression for Ryder's (1965) data. Partial correlation values (Type II, SAS Institute Inc. 1988) represent correlations between the entered variable and observed harvest, corrected for intercorrelations of the other variables with observed harvest. Simple Pearson correlation coefficients are uncorrected for correlations between entered variable and observed harvest.

Variable	Partial (corrected) correlation	Simple Pearson (uncorrected) correlation
Log_e area	0.845	0.974
Log_e volume	0.551	0.929
Log_e TDS	0.240	−0.023

Relationships among Model Variables

Partial correlation analysis indicates that surface area, volume, and TDS (in that order of strength) are independently correlated with fish harvest (Table 5). Partial correlation analysis helps correct for the effects of intercorrelations among variables, although some influence of the intercorrelations may remain.

Comparison of coefficients from partial and uncorrected correlation analysis illustrates the effect of intercorrelation on data interpretation (Table 5). Uncorrected (e.g. simple Pearson) correlations indicate that surface area and volume are equally correlated, and TDS is virtually uncorrelated, with fish harvest whereas the corrected analysis indicates that area, volume, and TDS are all correlated with harvest, but in descending order of strength. The erroneous interpretation, based on uncorrected correlations, occurs because of the strong intercorrelation between surface area and lake volume and is thus inappropriate for this analysis.

It is also clear from the multiple regression that morphometric and edaphic factors do not contribute equally to lake production. This model separates the contribution of the two components in predicting fish harvest. Morphometric factors (surface area and lake volume) account for 94.8 and 2.54%, respectively, of the variability in fish production. Edaphic factors (measured by TDS) account for only 0.64% of the variability when morphometric effects have been incorporated. Once again, it should be noted that in "atypical" conditions, volume and TDS contribute substantially to the accuracy of prediction.

Application of the MEM

It should be evident that parameters estimated in this model are a reflection of the data set chosen for analysis. In some ecoregions (or management regions), edaphic factors will play a greater role than in the primarily oligotrophic data set used here. Fisheries managers can expand on the original MEI data set with data based on their own management area. With the easy access of computer statistical software (e.g. SAS), there is no need to be encumbered by model parameters developed in an ecological region that differs from the management region of concern.

It should also be recognized that the most difficult component of developing a model data set is the estimation of yearly sustainable harvest. The original MEI data set was based on a sound, long-term estimate of sustainable yearly harvest, and that is why it will continue to be a primary data set for management purposes. Evaluation of future MEM refinements must clearly focus on the quality of the sustainable harvest estimates.

Approaches to Comparing Fish Production

A traditional use of the MEI was to compare potential fish production between lakes. Because the MEM directly provides estimates of annual fish harvest, the need for such comparisons is lessened. However, the model also makes clear the difficulty in deriving interlake comparisons. Four parameters, area, volume and TDS slope, and model intercept, can all vary between classes of lakes.

A deceptively simple approach is to reexpress the predicted annual harvest (along with estimated upper and lower confidence limits) in terms of standardized units of harvest per square metre (surface area) or harvest per cubic metre (lake volume). Because results can be divided by only one of these quotients, basin morphometry cannot be fully accounted for, so the cause for different yields per standard unit is not evident.

Perhaps a better approach would be to use multivariate (rather than simple multiple) regression techniques to test for differences in slope and intercept. Consider, for example, a single group of lakes where fishing pressure has changed. Annual-sustained harvest for the two time periods could be expressed as two separate dependent variables. Using multivariate regression, the significance of differences among slopes of the two time periods (e.g. low and high fishing pressure) could be tested (e.g. MTEST in the REG procedure of SAS). Results may be interpreted in the context of functional relationships between variables as a result of fishing pressure.

Alternatively, if two different groups of lakes are being compared (perhaps lakes with different fish community structure), a test of the hypothesis that community structure influences sustained annual harvest could be performed by including a dummy variable. Lake "a" would be assigned the value 0, lake "b" the value 1. The significance of the lake group variable can be determined and its percent contribution to explaining annual harvest estimated (see Goddard et al. 1987).

Interpretation of regression residuals can also aid in interpreting trends occurring among certain classes of lakes. For example, if regression analysis indicates that lakes with a certain community structure of fish consistently have predicted yields below the "average" regression line, analysis of residuals (difference of actual value from predicted value) will reveal this. Regression analysis can thus provide more insight into data relationships than the analysis of ratio data.

A Management Example

The MEM we have developed provides a valid statistical framework to approach fisheries management issues. The parameters derived using Ryder's (1965) data are most applicable to lakes similar to those within that data set. To illustrate how these parameters may change, we derive MEM parameters for two different Ontario fisheries management data sets.

The Ontario Ministry of Natural Resources developed a Strategic Plan for Ontario Fisheries and used the original MEI to partition yields for individual fish species (OMNR 1982). The index was applied to two management groups of lakes: sport and commercial fisheries. Data were taken from tables 1 and 3 of OMNR (1982). Some corrections in lake area were made to the sport fisheries data set (table 1 of OMNR 1982). Escanaba Lake was added to this data set, and several of the lakes are located in the United States.

The two groups of lakes differ with respect to MEM variables. The sport fisheries lakes are smaller and more fertile (mean area = 5267 ha, mean volume = 253 666·m^{-3}, TDS = 66.4 mg·L^{-1}) than the commercial lakes (mean area = 558 840 ha, mean volume = 60 938 827·m^{-3}, TDS = 57.7 mg·L^{-1}). As a consequence, the MEM parameters differ between the two groups of lakes. For the sport fisheries lakes, the derived model is

$$\text{(6)}\quad \text{Harvest} = \text{area}^{1.44}\cdot\text{vol}^{-0.49}\cdot\text{TDS}^{0.49}\cdot\text{EXP}^{4.49} \quad (r^2 = 0.84 \text{ and } p < 0.0001)$$

whereas for the commercial fisheries lakes, the model is

$$\text{Harvest} = \text{area}^{1.25}\cdot\text{vol}^{-0.26}\cdot\text{TDS}^{0.17}\cdot\text{EXP}^{3.00} \quad (r^2 = 0.98 \text{ and } p < 0.0001).$$

It is evident that TDS is much more important for predicting annual harvest in the smaller sport fisheries lakes than in the larger commercial fisheries lakes. Comparison of the observed sustained yield with predictions from the MEI and the MEM_r, based on Ryder's (1965) data set, and the MEM_s, based on the Sport Fisheries data set (Table 6), reveals that the MEM_s better fits the data than the two models based on Ryder's data set. Similarly, the MEM_c, derived from the commercial fisheries data set, provides the best fit to observed yield of the three models (Table 7). Thus, increased precision, at the sacrifice of generality, is gained by partitioning the data sets and deriving separate models for each group. The comparison of equations (4) and (6) also reveals sensitivity of the MEM_s parameters. For example, changes to the second decimal place of the exponents account for the differences in predicted yield for these equations in the sports fisheries data set (Table 6).

Outliers

Although the MEM, as developed from the data sets partitioned into sport and commercial fisheries, renders a more precise model of expected annual fish yield than the MEI, some lakes still remain as "outliers" to the multiple regression model, particularly Big Wolfe, Rice, Orr, and Escanaba sport fisheries lakes. We contacted fisheries biologists working on each of these lakes to see if particular cultural, ecological, or physiochemical factors may differ from the average in these lakes. Such information may provide insight into what additional variables or stratification may be required to broaden the generality or increase the precision of the MEM. Indeed, it is often the examination of outliers that leads us to a greater understanding of how systems function. The following three issues were identified:

(1) *Selective harvest and community structure*. In U.S. lakes, fishermen harvest, and keep, a greater diversity of fish than in Ontario. For example, in Escanaba Lake, WI (Kempinger and Carline 1977), and Big Wolfe Lake, MN (OMNR 1982), yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) are harvested at levels much higher than in the other lakes in the sport fisheries data set. Yellow perch is harvested at a higher rate in Rice Lake, ON (OMNR 1982). In addition to increasing yearly harvest, this selection also alters the predator/prey community structure, which in turn may affect population dynamics of other harvested species. In particular, one might speculate that high harvests of predatory yellow perch allows walleye populations to increase.

(2) *Phosphorus loading*. Higher than average phosphorus loading, from both natural and cultural influences, is associated with the higher harvests in Big Wolfe and Rice lakes.

Big Wolfe Lake received secondary treatment sewage from Bemidji, MN, via the Mississippi River (D. Schupp, pers.

TABLE 6. Comparison of observed yield ($kg \cdot ha^{-1} \cdot yr^{-1}$) for Ontario sport fisheries lakes ($n = 74$). Values are for actual observed yield, and predicted yield based on MEM for the sport fisheries data set (MEM_s), MEM based on Ryder's (1965) data set (MEM_r), and MEI based on Ryder's data set. Yield is used only for presentation of results and is obtained by dividing results of equations (6) (MEM_s), (5) MEM_r), and (4) (MEI) by lake area.

Lake	Observed	MEM_s	MEM_r	MEI
Tahoe	0.27	0.26	0.51	0.68
Chiblow-Denman	0.92	0.54	1.33	1.17
Flack	1.24	0.62	1.39	1.26
Kawagama	0.87	0.61	1.47	1.32
Joseph	0.84	0.63	1.49	1.39
Redstone	1.54	0.69	1.50	1.40
Lake of Bays	0.21	0.64	1.58	1.42
Rosseau	0.45	0.67	1.56	1.48
Little Quirke	0.33	0.81	1.76	1.55
Semiwite	0.76	0.84	1.76	1.58
Clearwater (MN)	0.25	0.84	1.86	1.63
Darragh	0.82	0.90	1.74	1.64
Bone	1.97	0.95	2.04	1.69
Opeongo	0.33	0.79	1.99	1.72
Happy Isle	0.28	0.94	1.87	1.81
Mississauga	0.93	0.98	1.87	1.88
Haliburton	0.62	0.97	1.94	1.92
Bernard	1.23	0.94	2.04	1.92
Temagami	0.46	0.84	2.10	1.93
Jimchrist	1.62	1.16	2.18	1.98
Lavieille	0.57	0.99	2.17	2.03
Drag	0.68	1.05	2.00	2.05
Pevensey	1.67	1.17	2.30	2.08
Gull	1.96	1.08	2.09	2.11
Mountain	1.74	1.19	2.18	2.18
Pickerel	2.59	1.18	2.52	2.22
Sturgeon	0.42	0.98	2.50	2.22
Merchant	0.66	1.25	2.54	2.31
Red 2 & 4	1.46	1.05	2.57	2.31
Spring	0.60	1.39	1.91	2.35
Red 1	1.08	1.14	2.56	2.37
N. Light	0.55	1.11	2.86	2.37
Eagle	1.41	1.27	3.00	2.44
Kashagawigamog	1.52	1.29	2.43	2.45
Kashe	2.14	1.29	3.17	2.45
Kirkness	0.47	1.25	3.16	2.53
Eagle	1.73	1.13	3.32	2.56
Peninsula	1.48	1.36	2.79	2.58
Doe	3.37	1.39	3.24	2.68
Lac des Mille Lacs	4.82	1.41	3.28	2.81
Redrock	0.41	1.58	3.20	2.85
Stormer	3.27	1.86	4.14	3.29
Wendigo	3.66	1.87	3.21	3.35
Davis	2.65	2.08	3.06	3.45
Gullrock Complex	2.50	1.66	3.90	3.47
Escanaba (WI)	19.70	2.09	3.91	3.50
Esnagi	3.44	1.76	4.01	3.53
Nipissing	3.85	1.62	4.88	3.75
Head	3.15	2.40	5.07	4.37
Vaughn	5.86	2.28	4.67	4.42
Cameron	3.80	2.39	4.50	4.43
Balsam	5.60	2.32	4.96	4.56
Coli	1.85	2.43	5.87	4.59
Four Mile	1.91	2.71	4.23	4.84
Gloucester Pool	2.83	3.12	5.04	5.66
Big Wolfe (MN)	15.12	3.41	4.69	5.83
Onaman	1.10	2.96	7.56	5.96
Andrusia (MN)	9.52	3.47	4.94	6.02
Cass (MN)	6.16	3.15	5.41	6.13
St. John	2.18	3.56	5.91	6.17
Sparrow	2.48	3.51	6.08	6.26
Severn	4.65	3.15	6.77	6.34
Sturgeon	6.50	3.42	6.65	6.49
Lower Buckhorn	5.04	3.79	6.75	6.74
Pigeon	4.58	3.58	7.31	6.85
Whitefish	8.97	3.85	8.34	7.11
Couchiching	1.65	4.14	7.41	7.79
Lovesick	8.61	4.85	7.80	7.87
Rice Lake	22.00	4.18	8.81	8.14
Dalrymple	2.41	5.61	9.48	9.70
Little	5.45	7.70	11.30	11.96
Orr	0.83	8.03	12.93	12.59
Scugog	3.10	7.06	13.81	12.88
Matchedash Bay	4.38	9.26	15.11	14.60

TABLE 7. Comparison of observed yield ($kg \cdot h^{-1} \cdot yr^{-1}$) for Ontario commercial fisheries lakes. Values are for observed yield, and predicted yield based on MEM for commercial fisheries data set (MEM_c), MEM based on Ryder's (1965) data set (MEM_r), and MEI model based on Ryder's data set. See Table 6 for further explanation of terms.

Lake	Observed	MEM_c	MEM_r	MEI
Superior	0.98	0.85	0.89	0.93
Lawrence	1.40	1.34	1.61	1.55
Michigan	1.80	1.11	1.48	1.63
Reindeer	2.34	1.34	2.00	1.66
Nipigon	0.95	1.20	1.55	1.66
Cree	1.04	1.37	2.13	1.67
Ontario	1.07	1.19	1.59	1.88
Huron	1.45	1.22	1.79	1.90
Wollaston	1.18	1.41	2.27	1.92
Atikwa	1.65	1.52	2.25	2.00
Athabasca	0.42	1.37	2.19	2.01
Lake of Bays	1.18	1.61	2.47	2.25
Eagle	2.08	1.75	3.32	2.56
Marchington	1.56	1.76	3.00	2.58
Nungessier	1.43	1.83	3.49	2.62
Penassi	1.27	1.83	3.13	2.64
Basket	2.21	1.84	3.37	2.71
Hooker	2.08	1.93	3.54	2.98
Gullrock	1.88	1.87	3.43	3.05
Wintering	2.07	1.98	3.70	3.10
Confusion	1.83	1.89	3.11	3.29
MacDowell	2.60	1.96	3.91	3.30
Cliff	1.68	1.91	3.25	3.36
Little Trout	2.09	1.98	3.74	3.36
Raggedwood	1.89	2.12	3.98	3.89
Weagamow	1.98	2.08	4.30	3.95
LaRonge	0.93	1.93	3.96	4.02
Clay	2.80	2.12	4.10	4.18
Windigo	2.27	2.12	4.33	4.20
Lake of the Woods	3.15	2.05	4.81	4.21

comm.), which resulted in higher phosphorus loading into the lake system. Rice Lake also has high phosphorus loading, due to its position on the drainage system. As the last lake on the Kawartha chain, it receives nutrients from the whole system, including upstream towns and agricultural land (D. Wood, pers. comm.). In addition, the lake has a high exchange rate (10 times per year), thus increasing the flow rate of nutrients through the system.

Big Wolfe and Escanaba lakes are located further south than most of lakes in the data set, thus having a longer growing season. Also, the land in Wisconsin and Minnesota is generally more fertile than that on the Canadian Shield, where many of the lakes in the data set are located. This factor may also lead to higher phosphorus loading.

(3) *Habitat degradation*. Orr Lake is a headwater lake that experiences noxious winter kills and algal blooms. The lake

has lower than expected yields with fish growth below average and there is almost complete development (degradation) of shoreline habitat (R. Craig, pers. comm.). Water skiing occurs overtop the bass spawning grounds, and this may impede natural reproduction. These factors may interact to produce a fish community that is less desirable as a sport fishery, thus resulting in lower than expected yields.

Clearly, these three factors may potentially influence the expected harvest of fish from a lake, and a reevaluation of data sets would demonstrate how strong (and significant) these relationships are. Two statistical approaches for dealing with this variability have already been discussed: (i) partition the data sets (just as we partitioned the sport and commercial fisheries) to increase the precision of the model and (ii) include additional variables in the multiple regression (e.g. phosphorus loading) to increase the generality of the model.

Past performance of these additional factors cannot be used to judge their potential performance in the MEM. For example, phosphorus loading alone may be unable to account for a large proportion of the variability in annual harvest, but as a variable in the multiple regression, it may be able to account for lakes that remain outliers in the current model. Finally, it should be clear that any statistical model based on a geographically restricted set of lakes, with particular cultural and environmental influences, will not be suitable for predicting expected annual harvests of fish existing under different conditions. However, with proper partitioning or stratification of the data set, and with selection of meaningful variables, models can be derived that will allow managers to predict expected harvests with acceptably high (and measurable) levels of confidence.

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References

BAJDIK, C. D., AND D. C. SCHNEIDER. 1991. Models of the fish yield from lakes: does the random component matter? Can. J. Fish. Aquat. Sci. 48: 619–622.

CHATTERJEE, S., AND B. PRICE. 1977. Regression analysis by example, John Wiley and Sons, New York, NY. 228 p.

CHRISTIE, G. C., AND H. A. REGIER. 1988. Measures of optimal thermal habitat and their relationships to yields for four commercial fish species. Can. J. Fish. Aquat. Sci. 45: 301–314.

COLBY, P. J., AND S. J. NEPSZY. 1981. Variation among stocks of walleye (*Stizostedion vitreum vitreum*): management implications. Can. J. Fish. Aquat. Sci. 38: 1814–1831.

DOWNING, J. A., C. PLANTE, AND S. LALONDE. 1990. Fish production correlated with primary productivity, not the morphoedaphic index. Can. J. Fish. Aquat. Sci. 47: 1929–1936.

GODDARD, C. I., D. H. LOFTUS, J. A. MACLEAN, C. H. OLVER, AND B. J. SHUTER. 1987. Evaluation of the effects of fish community structure on observed yields of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 44(Suppl. 2): 239–248.

GREEN, R. H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley and Sons, Inc., Toronto, Ont. 257 p.

JACKSON, D. A., H. H. HARVEY, AND K. M. SOMERS. 1990. Ratios in aquatic sciences: statistical shortcomings with mean depth and the morphoedaphic index. Can. J. Fish. Aquat. Sci. 47: 1788–1795.

KEMPINGER, J. J., AND R. F. CARLINE. 1977. Dynamics of the walleye (*Stizostedion vitreum vitreum*) population in Escanaba Lake, Wisconsin, 1955–72. J. Fish. Res. Board Can. 34: 1800–1811.

LEACH, J. H., L. M. DICKIE, B. J. SHUTER, U. BORGMANN, J. HYMAN, AND W. LYSACK. 1987. A review of methods for prediction of potential fish production with application to the Great Lakes and Lake Winnipeg. Can. J. Fish. Aquat. Sci. 44(Suppl. 2): 471–485.

OGLESBY, R. T. 1977. Relationships of fish yield to lake phytoplankton standing crop, production, and morphoedaphic factors. J. Fish. Res. Board Can. 34: 2271–2279.

1982. The MEI Symposium — overview and observations. Trans. Am. Fish. Soc. 111: 154–164.

OGLESBY, R. T., J. H. LEACH, AND J. FORNEY. 1987. Potential *Stizostedion* yield as a function of chlorophyll concentration with special reference to Lake Erie. Can. J. Fish. Aquat. Sci. 44(Suppl. 2): 166–170.

OMNR. 1982. Strategic Planning for Ontario Fisheries: partitioning yields estimated from the morphoedaphic index into individual species yields. Report of SPOF Working Group Number Twelve, Ontario Ministry of Natural Resources, Toronto, Ont.

PRAIRIE, Y. T., AND D. F. BIRD. 1989. Some misconceptions about the spurious correlation problem in the ecological literature. Oecologia 81: 285–288.

REGIER, H. A., J. A. HOLMES, AND D. PAULY. 1990. Influence of temperature changes on aquatic ecosystems: an interpretation of empirical data. Trans. Am. Fish. Soc. 119: 374–389.

REMPEL, R. S., AND J. C. H. CARTER. 1987. Modelling the effects of accelerated development and reduced fecundity on the population dynamics of aquatic Diptera. Can. J. Fish. Aquat. Sci. 44: 1737–1742.

RYDER, R. A. 1965. A method for estimating the potential fish production of north-temperate lakes. Trans. Am. Fish. Soc. 94: 214–218.

1982. The morphoedaphic index-use, abuse and fundamental concepts. Trans. Am. Fish. Soc. 111: 154–164.

SAS INSTUTUTE INC. 1988. SAS/STAT® user's guide, release 6.03 edition. SAS Institute Inc., Cary, NC. 1028 p.

SCHLESINGER, D. A., AND H. A. REGIER. 1982. Climate and morphoedaphic indices of fish yields from natural lakes. Trans. Am. Fish. Soc. 111: 141–150.

SCHNEIDER, D. C., AND R. L. HAEDRICH. 1989. Prediction limits of allometric equations: a reanalysis of Ryder's morphoedaphic index. Can. J. Fish. Aquat. Sci. 46: 503–508.

YOUNGS, W. D., AND D. G. HEIMBUCH. 1982. Another consideration of the morphoedaphic index. Trans. Am. Fish. Soc. 111: 151–153.

Effects of Time of Day, Water Temperature, and Water Velocity on Swimming by Postlarvae of the American Lobster, *Homarus americanus*

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Rooney, P., and J. S. Cobb. 1991. Effects of time of day, water temperature, and water velocity on swimming by postlarvae of the American lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 48: 1944–1950.

Swimming speeds and swimming behavior of laboratory-reared and wild postlarvae (larval stage IV) of the American lobster, *Homarus americanus*, were observed in tanks of still water and in a linear flume. Mean swimming speeds ranged from about 7 $cm \cdot s^{-1}$ in the "claws-apart" swimming mode to 13.2 $cm \cdot s^{-1}$ in the streamlined, "claws-together" mode. In the flume, the total time spent swimming during a 30-min test period decreased as water velocity increased from 8 to 14 $cm \cdot s^{-1}$. The time spent swimming was greater during the day than at either dusk or night. Postlarvae spent very little time swimming at 15°C but significantly more at 21°C. Wild postlarvae were larger, heavier, and swam more rapidly than laboratory-reared postlarvae. Rapid, directional swimming by postlarval lobsters may play a role in determination of distribution and recruitment to benthic populations.

On a observé la vitesse et le comportement de nage de sujets post-larvaires (stade larvaire IV) du homard d'Amérique, *Homarus americanus*, sauvages et élevés en laboratoire, qui avaient été placés dans des bassins d'eau non renouvelée et dans une goulotte de forme linéaire. La vitesse moyenne de nage était d'environ 7 $cm \cdot s^{-1}$ lorsque les sujets nageaient « les pinces écartées », à 13,2 $cm \cdot s^{-1}$ lorsqu'ils nageaient « les pinces jointes », dans une forme davantage hydrodynamique. Dans la goulotte, la durée totale de nage durant une période d'essai de 30 min diminuait à mesure que la circulation de l'eau était accélérée de 8 à 14 $cm \cdot s^{-1}$. Le temps consacré à la nage le jour était supérieur au temps consacré à la nage à la brunante ou la nuit. Les sujets post-larvaires passaient très peu de temps à nager lorsque l'eau était à 15°C, mais en passaient significativement plus lorsque l'eau était à 21°C. Les sujets sauvages étaient plus gros et plus lourds, et nageaient plus rapidement que les sujets élevés en laboratoire. La nage rapide et orientée de homards post-larvaires peut avoir un rôle dans la détermination de la distribution et du recrutement dans des populations benthiques.

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The postlarva (stage IV) of the American lobster, *Homarus americanus*, is a transitional stage between the planktonic mysis form of the first three larval stages and the benthic juvenile phase. Although very similar in appearance to juveniles and adults, the postlarvae are markedly different in behavior. They generally are found in the upper metre of the water column (Fogarty 1983; Harding et al. 1987; Hudon et al. 1986) and are capable of rapid, directional swimming (Cobb et al. 1989; Ennis 1986a; Herrick 1909; Smith 1873). Laboratory-reared postlarvae were able to maintain position against a current of 9 $cm \cdot s^{-1}$ in a flume (Ennis 1986a). Field observations of wild, free-ranging postlarvae gave an average swimming speed of 18 $cm \cdot s^{-1}$ (Cobb et al. 1989).

Considerations of larval recruitment in *H. americanus* generally incorporate the assumption that larval distributions are determined passively and can be approximated by patterns of residual drift. However, evidence is now emerging that larval behavior may affect distribution patterns (see review by Ennis 1986b). Early larval stages appear to migrate vertically in the upper layers over deep water (i.e. offshore) (Harding et al. 1987) and thus could affect rate, and possibly direction, of drift. The postlarvae appear to be present in the surface layer at all times, but their directional swimming behavior (Cobb et al. 1989), if sustained, could allow movement on the order of tens of kilometres. Caddy (1979) and Hudon et al. (1986) both have observed changes in the centers of distribution of larval stages that could not be explained fully by patterns of surface residual drift. Caddy (1979), Hudon et al. (1986), and Cobb et al. (1989) suggested that horizontal swimming by the postlarvae may affect distribution and thus recruitment patterns. Clearly, further study of the swimming behavior is warranted.

We measured speed of lobster postlarvae swimming freely in a large tank. We also examined swimming speed and behavior of postlarvae in a flume under several treatment conditions of light, temperature, and water velocity.

Methods

The postlarvae used in this study were either reared at the Massachusetts State Lobster Hatchery (laboratory-reared) or captured in Block Island Sound (wild). Larvae were transported from the hatchery to the University of Rhode Island in chilled seawater and held 4–10 d before testing. Wild postlarvae also were transported in chilled seawater and held 2–5 d before testing. The animals were maintained in planktonkreisels designed for larval lobsters (Hughes et al. 1974). The water was maintained at one of three water temperatures (21, 18, 15°C) by chilling the 21°C incoming seawater. A light-tight enclosure

and timed lights provided a light regime of three intensities: night (20:30–05:30; 0–01 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the water surface), dawn and dusk (05:30–08:30; 17:30–20:30; 0.5 $\mu E \cdot m^{-2} \cdot s^{-1}$), and day (08:30–17:30; 4.5 $\mu E \cdot m^{-2} \cdot s^{-1}$). The postlarvae were fed frozen adult *Artemia* (San Francisco Brand) twice daily.

The carapace length and molt stage were determined for each postlarva after its trial in the flume. Molt stage was determined using the criterion of setal development (Sasaki 1984). Only postlarvae that were in intermolt (stage C) or early premolt (stage D_0 or D_1) were used. Ennis (1986a) showed that newly molted postlarvae (stage A or B) were not as capable swimmers as older animals.

Still Water Observations

Forty laboratory-reared postlarvae were tested at the Massachusetts State Lobster Hatchery on 13 May 1987 in a 0.9 × 2.1 m indoor tank with a water depth of 0.28 m at 21°C. Postlarvae were released into the tank and swimming behavior was recorded on videotape from approximately 2 m above the water surface. Each animal was removed from the tank and its molt stage determined immediately after videorecording. Wild animals were tested at the University of Rhode Island on 19 July 1987 in a 1.2 × 3.1 m outdoor tank with a water depth of 0.3 m. Nine postlarvae were tested in water temperature of 20°C. In both tanks, a 2-cm grid laid on the bottom provided reference for later velocity measurements. There was no water flow into the tanks and no perceptible water movement during the test.

Flume Observations

Two modes of swimming have been described (Botero and Atema 1982; Cobb et al. 1983). In the first, the postlarva assumes a streamlined posture, claws directed anteriorally ("claws-together"); in the second, the claws are directed anterolaterally, spread apart ("claws-apart"). Swimming speeds of animals in both swimming modes were determined from the tapes of laboratory-reared and wild animals using stop-motion analysis. A stopwatch function on the videocamera recorded time to 0.1 s. We only measured swimming paths that were straight and lasted more than 2.0 s. For some animals, more than one straight path was available; in those cases, we calculated a mean velocity.

Changes in postlarval swimming behavior under different conditions of light, water velocity, and water temperature were observed in a linear flume. Tests on the effects of time of day and water velocity were performed on laboratory-reared postlarvae in 1987, and the effects of water temperature and time of day were tested on wild animals in 1988.

A flume similar to the one described by Ennis (1986a) was used. It consisted of an open oval channel 20 × 20 cm with two linear observation chambers of 1 m length on either side of the oval (Fig. 1). We found no significant difference (ANOVA, $p = 0.458$) between the total time spent swimming by postlarvae in observational chamber 1 and in chamber 2, so the data were pooled. A 1-cm grid was engraved in the bottom of each chamber so the position of the postlarva could be determined. Water depth was maintained at 18 cm. The water was moved by an outboard engine propeller attached to an electric variable-speed motor. Stacked hexagonal aluminum tubes, 3 mm in diameter, were placed upstream and downstream of each observational chamber to ensure that the water flow in each chamber was laminar. Laminar flow was observed using dyed milk injected in various positions of each observation chamber prior to testing (Vogel 1981). Water velocity was continuously monitored during testing using a Marsh-McBirney portable water current meter.

The experiment in 1987 determined the swimming behavior of laboratory-reared postlarval lobsters at each of three velocities, three times of day, and three temperatures in a blocked statistical design. Ten animals were tested in each treatment combination. Actively swimming, apparently healthy animals were selected from the planktonkreisels and placed in a holding funnel for testing. Because the temperature treatment was not properly replicated, experiments on the effect of temperature were repeated in 1988. We report only experiments performed at 18°C in 1987.

Two postlarvae were tested at a time, one in each observational chamber. Each animal was used only once. At the beginning of each trial, two postlarvae were selected haphazardly from the holding funnel and one placed in each chamber 10 cm from the upstream screen, facing into the current. The behavior of each animal was observed continuously for 30 min. Swimming position (claws-apart, claws-together) and position in the flume (in 1-cm increments) were recorded. Time and behavioral state were recorded whenever behavior changed or at 2-min intervals if no change occurred. A total of 10 animals were tested in each of the experimental conditions.

Postlarvae showed a positive rheotaxis, orienting into the current in the flume. They responded to the flowing water by moving in one of three ways: they swam against the current, they went to the bottom of the flume and "swim/crawled," or crawled on the bottom. Here, we report only on the time spent swimming. In determining time spent swimming, no distinction was made between claws-apart and claws-together modes.

Water Velocity and Time of Day

The effects of water velocity and of time of day on amount of time postlarvae spent swimming were tested by subjecting laboratory-reared postlarvae to one of three water velocities and at one of three times of day. The animals were tested at 8, 10, and 12 $cm \cdot s^{-1}$ between the hours of 10:00 and 15:00 (day, light intensity 2.0 $\mu E \cdot m^{-2} \cdot s^{-1}$), between 18:00 and 21:30 (evening, 0.6 $\mu E \cdot m^{-2} \cdot s^{-1}$), and between 23:00 and 01:30 (night, 0.3 $\mu E \cdot m^{-2} \cdot s^{-1}$). To provide the different light intensities, a red light was on at all times and provided the only illumination at night, two 25-W incandescent bulbs were added for evening, and two 25-W cool white fluorescent bulbs covered with a diffuser simulated daylight. Water temperature was 18°C.

Water Temperature

In 1988, the effect of water temperature on the time spent swimming was tested by observing wild postlarvae at each of three temperatures: 15, 18, and 21°C during the day and night. The temperature range chosen represents summer temperatures likely to be experienced by postlarvae in southern New England waters. The animals were acclimated in the planktonkreisels test temperature for 2–5 d before testing. The postlarvae tested in the third replicate of 15°C were acclimated at 18°C due to failure of the chiller system. There was no significant difference among these three replicates, so all of the data were used.

Postlarvae were tested only during the day and night because the 1987 data showed no significant difference between the times the postlarvae spent swimming during the evening and night. Water velocity was 14 $cm \cdot s^{-1}$. Three sets of 10 animals

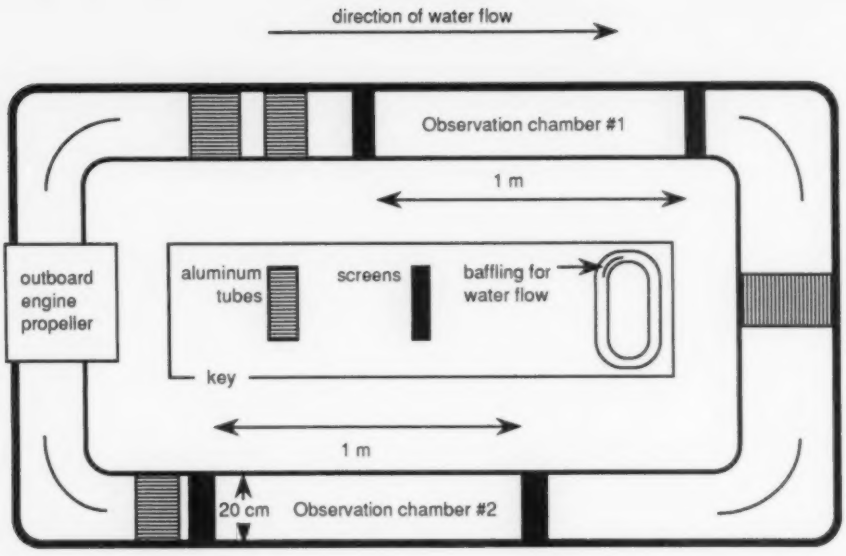

FIG. 1. Linear flume used for behavioral studies of the American lobster postlarvae. Total size of the flume is 244 × 91 cm.

TABLE 1. Mean and standard deviation of swimming speeds of laboratory-reared and wild American lobster postlarvae in two swimming modes, tested in large, still water tanks.

	Mean (SD) ($cm \cdot s^{-1}$)	N	Range
Laboratory-reared			
Claws-apart	7.1 (1.4)	24	4.2–9.5
Claws-together	10.7 (1.9)	16	7.4–14.4
Wild			
Claws-apart	6.6 (1.8)	2	5.3–7.9
Claws-together	13.2 (3.1)	7	9.2–16.7

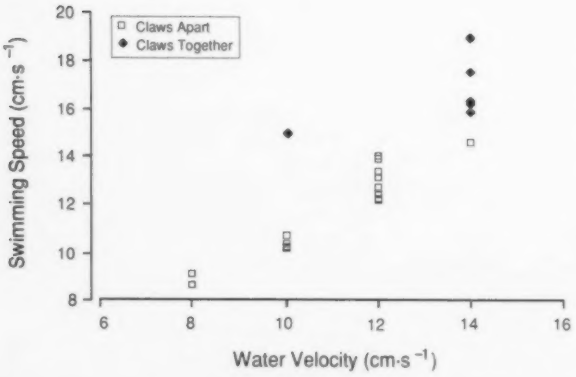

FIG. 2. Maximum swimming speeds measured over straight-line paths of greater than 2 s duration of laboratory-reared and wild American lobster postlarvae in two swimming modes tested in a linear flume at 18°C in 1987 and 1988.

TABLE 2. Mean and standard deviation of carapace length and wet weight of laboratory-reared and wild American lobster postlarvae.

	N	Mean carapace length (mm)	Mean wet weight (g)
Laboratory-reared	145	3.88 (0.21)	0.03 (0.01)
Wild	61	4.84 (0.27)	0.05 (0.01)

were tested at each temperature during both times of the day for a total of 180 postlarvae.

Results

Swimming Speeds

Still water observations

The postlarvae of both laboratory-reared and wild animals swam faster in the claws-together mode than in the claws-apart mode (ANOVA, $p = 0.0001$) (Table 1). Laboratory-reared postlarvae swam significantly more slowly (7.1 $cm \cdot s^{-1}$) in the claws-apart mode than in the claws-together mode (10.7 $cm \cdot s^{-1}$) (t-test, $p = 0.0001$). Wild postlarvae swam at a mean speed of 6.6 $cm \cdot s^{-1}$ in the claws-apart mode and 13.2 $cm \cdot s^{-1}$ in the claws-together mode. The swimming speed of only two wild animals was measured in the claws-apart mode, so this estimate should be regarded with caution. Wild postlarvae swam significantly faster than the laboratory-reared postlarvae in the claws-together mode (t-test, $p = 0.03$).

Flume observations

Observations of postlarvae in the flume allowed us to determine swimming speeds of some postlarvae. The time to move a known distance over the bottom in an upstream direction was noted and the resultant velocity was added to the water velocity. Figure 2 represents maximum swimming speeds thus measured during the day at 18°C in both 1987 and 1988. In the claws-

TABLE 3. Mean and standard deviation of total time (seconds) spent swimming by laboratory-reared American lobster postlarave in a linear flume at three water velocities and at three times of day. $N = 10$ for each treatment; test time = 1800 s.

Velocity	Day	Evening	Night
8 cm·s^{-1}			
Mean	640.2	357.2	310.0
SD	664.4	599.1	595.4
10 cm·s^{-1}			
Mean	546.1	109.1	49.4
SD	687.8	163.0	71.6
12 cm·s^{-1}			
Mean	161.1	11.1	6.2
SD	230.9	7.3	4.9

TABLE 4. Mean and standard deviation of time (seconds) spent swimming by wild American lobster postlarvae in the linear flume at two times of day and at three temperatures. $N = 10$ for each mean; test time = 1800 s.

Temperature	Day	Night
15°C	11.1 (15.6)	10.8 (18.1)
18°C	133.1 (277.0)	15.5 (18.2)
21°C	212.5 (332.9)	20.4 (25.9)

apart swimming mode, the maximum speed approximated the water velocity, while in the claws-together mode, the maximum swimming speed exceeded water velocity. The number of animals whose swimming speeds we were able to measure in the flume was small; thus, we present no statistical treatment of the data.

Laboratory-reared postlarvae were significantly smaller in both carapace length and net weight than those caught in the field (*t*-tests, $p = 0.0001$) (Table 2). Carapace length measurements were available for some flume-tested animals. We compared swimming speeds measured in the flume of the laboratory-reared and wild postlarvae after expressing the velocity of each individual in terms of its carapace length. In the 18°C day treatment, laboratory-reared animals swam at a mean of 27.1 carapace lengths·s^{-1} ($N = 8$, SD = 5.5), while wild animals swam at a mean of 26.8 carapace lengths·s^{-1} ($N = 8$, SD = 2.4) in the claws-apart swimming mode. In the claws-together swimming mode, one laboratory-reared postlarva swam at 33.3 carapace lengths·s^{-1} and the wild postlarvae swam at a mean of 36.5 carapace lengths·s^{-1} ($N = 5$, SD = 3.5).

Time Spent Swimming

Water velocity and time of day

A two-way ANOVA showed that the mean total amount of time spent swimming during the 30-min test (T) by lobster postlarvae decreased as water velocity increased (ANOVA, $p = 0.012$) and that they swam more during the day than at night (ANOVA, $p = 0.004$) (Table 3). There was no significant interaction between statistical effects of time of day and water velocity.

In the 1987 tests, the mean total swimming time of laboratory-reared postlarvae during the day, pooled over all water velocities ($T = 449.1$ s), was significantly greater than the mean total swimming time during the evening ($T = 180.0$ s) and night ($T = 121.9$ s). There was no difference in the mean

TABLE 5. Mean number and standard deviation per trial of impingements of wild American lobster postlarvae on the downstream screen in a linear flume at two times of day and at three temperatures.

	Day impingements			Night impingements		
Temperature	*N*	Mean	SD	*N*	Mean	SD
15°C	29	4.7	3.9	30	3.5	3.0
18°C	30	6.3	3.6	29	4.6	2.5
21°C	30	9.0	5.2	30	5.6	4.3

total swimming time of the postlarvae between evening and night (ANOVA, followed by Duncans multiple range test, $p < 0.05$). Mean total swimming time pooled over all times of day was highest at a water velocity of 8 cm·s^{-1} ($T = 456.7$ s), intermediate at 10 cm·s^{-1} ($T = 234.9$ s), and lowest at 12 cm·s^{-1} ($T = 59.5$ s). The Scheffe multiple comparison test indicated that the means at water velocities of 8 and 12 cm·s^{-1} were significantly different.

There was an increase in the amount of time spent swimming at increased water temperatures. At 15°C and a water flow of 14 cm·s^{-1} the postlarvae swam very little. They swam approximately 7% of the time at 18°C and 12% at 21°C at the same flow rate. At lower water velocities, the postlarvae spent more time swimming; at 18°C and 8 cm·s^{-1}, swimming time occupied nearly 36% of the test time (640 s) (Table 3). There was a significant interaction between temperature effects and replicate in the ANOVA which we cannot explain other than by large variations among individual responses. There also was a significant interaction between effects of temperature and time of day which could be attributed to the postlarval response at 15°C, where there was no significant difference in total time spent swimming between day and night (Table 4).

Some animals did not move after being placed in the flume. The percentage of postlarvae that did not move was low during the day (13.3%), intermediate during the evening (36.7%), and highest at night (53.3%).

Water temperature and time of day

Preliminary data taken in the summer of 1987 suggested that water temperature and the amount of time the laboratory-reared postlarvae spent swimming were positively correlated. We investigated this further in the summer of 1988 using wild postlarvae at three temperatures at two times of day. Three replicates were done in each treatment combination.

The behavior of one postlarva was different from all others tested. This animal swam most of the 30-min observational period at 15°C during the day, while the mean total swimming time of the other postlarvae in this treatment was 11 s. When this animal's swimming time was removed from the analysis, the effect of temperature was significant (ANOVA, $p = 0.006$). The animal was considered an outlier (a single extreme variate, as calculated according to Sokal and Rohlf 1969) and the data from it were not included in subsequent analyses.

A three-factor ANOVA showed significant effects of both temperature ($p = 0.004$) and time of day ($p = 0.0001$) but not of replicates ($p = 0.632$). There was a significant interaction between temperature and replicate ($p = 0.023$) as well as between temperature and time of day ($p = 0.011$). Total time spent swimming was significantly greater at 21°C than at 15°C (ANOVA, $p = 0.011$) (Table 4). Total swimming time at 18°C was not significantly different from total swimming time at 15

or 21°C. There was an increase in time spent swimming during the day with increased temperature. The wild postlarvae also swam more during the day than at night (ANOVA, $p = 0.002$). However, at the lowest temperature, there was no significant difference in their total swimming time because the postlarvae did not swim much in either the day or night (Table 4).

If an animal stopped swimming for more than a few seconds, the current carried it downstream to the end of the flume where it would come in contact with the screen. There was a significant effect of both temperature and time of day treatments on the frequency of these impingements (two-factor ANOVA, $p < 0.0005$ for time of day, $p < 0.0001$ for temperature). The mean number of impingements was lowest at 15°C and at night (Table 5).

Discussion

The postlarval stage of *H. americanus* has long been known as a capable swimmer. Over 100 yr ago, Smith (1873, p. 374) noted that "In this stage the young lobsters swim very rapidly by means of the abdominal legs (pleopods) . . . They appear to live a large part of the time at the surface . . . and were often seen swimming about among other surface animals." Nevertheless, the significance of postlarval swimming has been overlooked until recently (Ennis 1986a, 1986b; Cobb et al. 1989). Rapid, directional swimming could, along with advective processes, affect horizontal distribution patterns and thus patterns of recruitment to the benthic stage. Laboratory experiments are needed to understand factors affecting swimming behavior (Sulkin 1986). In this study, we examined the influence of several environmental factors on swimming speeds. Postlarvae swam more during the day than they did at night. Water temperature was positively correlated with the amount of time spent swimming in the flume. As water velocity increased, the time spent swimming decreased, but the postlarvae were able to match or surpass all the water velocities with which they were challenged. Additionally, there was a difference in the swimming speeds in the two different modes, the "claws-apart" mode being slower than the "claws-together" mode.

The two modes of swimming appear to have different behavioral significance. When the postlarvae were in the streamlined, claws-together mode, they appeared to disregard everything else. If they encountered an object or another animal, they turned and swam around it. The claws-apart mode was used when investigating objects or feeding. We observed many postlarvae in the claws-together mode swimming around the edge of the planktonkreisels prior to feeding. After we placed *Artemia* in the tank, many animals would change from the claws-together mode to the claws-apart mode, catch *Artemia*, and remain in the claws-apart mode while feeding. Botero and Atema (1982) noted that the claws-together mode was used for fast and directional swimming and the claws-apart mode was observed when the lobsters were "relaxing" in the water column, moving their pleopods slowly and showing no directionality of movement. Cobb et al. (1983) described the two modes of swimming similarly but added that the claws-apart mode was used when the animal was investigating or picking at the surface in what appeared to be feeding motions.

The swimming speeds, when in the claws-apart mode, of postlarvae tested in the flume tended to match the water velocity with which they were challenged. The few measurements of swimming speed in the claws-together mode made in the flume were somewhat higher than the water velocity. This supports the suggestion that the claws-apart mode is more an investigatory and position-holding behavior, while the claws-together mode is for going places. Although we did not feel it was appropriate to make statistical comparisons between the flume and still water swimming speeds, it is noteworthy that the maximum velocities were measured in the flume. Measuring swimming speed in flowing water may provide more accurate estimates of maximal swimming capability of the postlarvae.

The swimming speeds reported here are lower than those of wild, free-ranging postlarvae we measured at sea (Cobb et al. 1989). Eighteen postlarvae observed swam at 7–24 $cm \cdot s^{-1}$ (mean = 18 $cm \cdot s^{-1}$). Ennis (1986a) challenged postlarvae in a flume with water velocities up to 11.5 $cm \cdot s^{-1}$; they swam against 9 $cm \cdot s^{-1}$ flows but not 11.5 $cm \cdot s^{-1}$ flows. The larvae of other lobsters also swim rapidly. Phillips and Olsen (1975) measured the puerulus stage of *Panulirus cygnus* swimming at speeds of up to 46 $cm \cdot s^{-1}$ in the field, Calinski and Lyons (1983) found speeds of up to 9 $cm \cdot s^{-1}$ in *P. argus* pueruli, and *P. interruptus* puereli were found to swim at speeds of 6–9 $cm \cdot s^{-1}$ (Serfling and Ford 1975).

We found that the wild postlarvae swam faster in our tests than did the laboratory-reared postlarvae. This may be due to a size difference. Laboratory-reared postlarvae were smaller and weighed less than wild postlarvae. Decreasing food availability or poor nutritional quality increase development time and decrease survival and growth in lobster larvae (Carlberg and Van Olst 1976; Eagles et al. 1986; Templeman 1936). The laboratory-reared postlarvae were reared under crowded conditions and were fed frozen adult *Artemia* (M. Syslo, Massachussetts State Lobster Hatchery, pers. comm.). Possibly, the nutritional state of the wild postlarvae was better than that of the laboratory-reared postlarvae and resulted in the larger sizes and faster swimming speeds we observed. Generally, the sizes of postlarvae captured at sea are larger than those reared in the laboratory (see table 1 in Hudon and Fradette 1988). If swimming speed is scaled to body size (i.e. carapace lengths per second rather than centimetres per second), then the smaller, laboratory-reared individuals appear to be as capable swimmers as the wild ones. Nevertheless, in the 1988 experiments, we chose to use only wild postlarvae both because of their faster swimming and because they probably had been exposed to a wider range of temperatures during development.

Responses to the flume environment were very different among individual postlarvae. A total of 560 animals were used in the flume study. Some, when placed in the flume, would drift to the downstream screen and not move for the 30-min trial, while others would swim as soon as they were placed in the flume and continue for the whole trial. There were many variations in between, leading to the high standard deviations seen throughout this study.

Although we used a flume similar to the one used by Ennis (1986a) in his study on the swimming of American lobster larvae, it is difficult to compare our results with his, since our data were collected under different conditions. His water velocities ranged from 0 to 11.5 $cm \cdot s^{-1}$, and he recorded the position and orientation of larvae at 30-s intervals. In contrast, we challenged postlarvae with water flows of 8–14 $cm \cdot s^{-1}$ and recorded behavioral data continuously. Nevertheless, some of our results are comparable. In the study by Ennis, the older postlarvae were swimming in 32% of his observations at 9 $cm \cdot s^{-1}$, and no swimming occurred at 11.5 $cm \cdot s^{-1}$. In our study at 18°C in 1987, postlarvae swam 36% of the time at 8 $cm \cdot s^{-1}$ and 9% of the time at 12 $cm \cdot s^{-1}$. E. P. Ennis (pers.

comm.) reported that the water temperature in his experiments was 14–16°C which was lower than the temperatures we used, possibly decreasing the swimming time of his postlarvae. The reason for an effect of temperature on the proportion of time spent swimming is not clear to us.

Swimming in a flume such as the one we used is caused by a rheotactic response on the part of the postlarvae. Hadley (1906) induced swimming in *H. americanus* postlarvae by rotating vertical stripes outside a circular test chamber and concluded that rheotaxis may be attributed to optical stimuli. We do not know whether the postlarvae in our flume were orienting into the current per se or if they were responding to a combination of stimuli, including optical and tactile, that kept them oriented into the current. At sea, swimming at or near the surface may provide cues, or external reference points (i.e. the sun), for swimming oriented into a current. Nevertheless, we agree with Ennis (1986a) that it is not obvious what role rheotaxis may play in the larval recruitment mechanism.

The postlarvae swam more during the day than at night. Adult lobsters are nocturnal, and we expected the postlarvae to exhibit the same tendency. Postlarval lobsters could use some orientation cue during the day that is not present at night. In the darkened flume, visual cues for rheotactic orientation were missing, or nearly missing. The lower frequency of swimming may be a reflection of this. Also, the postlarvae, when impinged on the downstream screen at night, made fewer attempts to get off the screen and were more likely to remain there (resulting in fewer impingements) (Table 5). This suggests that the motivation for swimming is indeed lower at night. Why should lobster postlarvae swim more during the day? One possibility is that they use a visual orientation cue, such as the sun, to guide their directional swimming. Bainbridge and Waterman (1957) found the littoral mysid *Mysidium gracile* was capable of orientating relative to the polarized plane of light. They also found that the littoral prawn *Palaemon northropi* swims on a relatively straight horizontal course for appreciable distances at sea. Many other arthropods use celestial cues for orientation; the daytime preference for swimming by *H. americanus* postlarvae may suggest that they too use celestial cues for orientation.

From our results, we would expect postlarval lobsters in the sea to swim most during daylight hours and at warmer temperatures. In addition to knowing swimming speed and proportion of time spent swimming under different conditions, it would be useful to know how far a postlarva is capable of swimming. In a linear flume, if an animal swims more slowly for a period of time, or stops for more than a few seconds, impingement on the downstream screen occurs, which must bias the results. Nevertheless, comparisons of performance at various times of day, water velocities, and water temperatures can indicate relative capacities for swimming under different environmental conditions.

At sea, animals swimming at an average of 18 $cm \cdot s^{-1}$ (Cobb et al. 1989) could travel considerable distances if there were sufficient energy reserves. Studies of energetics indicate that swimming in Antarctic krill, *Euphausia superba*, is little, if any, more expensive than hovering (Kils 1982). Sasaki et al. (1986) showed that lipid storage by lobster postlarvae provided up to 5 d of metabolic reserves. Our data show that postlarvae swim considerably more during the day than at night. If we assume that a postlarva swims 12 $h \cdot d^{-1}$ for 5 d at 18 $cm \cdot s^{-1}$, it could traverse about 39 km.

If suggestions that behavior of postlarvae is a determinant of distribution are shown to be correct, then the behavior we have explored is a mechanism that may play an important part in the larval recruitment process. Clearly, directionality of swimming is critical to the process as well. Directional swimming has been suggested to be important in the cross-shelf movement by the puerulus of *P. cygnus* (Phillips 1981) and also may play a role in the recruitment mechanism of other spiny lobsters. Cobb et al. (1989) observed the swimming direction of lobster postlarvae in Block Island Sound and found a high proportion (66%) to be swimming in a northerly and easterly direction. Evidence from many species of estuarine invertebrates indicates that active changes in vertical distribution can result in differential horizontal advection leading to retention in estuaries (Epifanio 1988) or return to the locality of origin (Phillips and McWilliam 1986). It is also clear that the megalopal or postlarval stages of some crustaceans are very capable swimmers in the horizontal plane. Directional swimming by postlarvae may be an important factor in larval recruitment and should be given consideration along with the effects of vertical migration.

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References

BAINBRIDGE, R., AND T. H. WATERMAN. 1957. Polarized light and the orientation of two marine crustacea. J. Exp. Biol. 34: 342–364.

BOTERO, L., AND J. ATEMA. 1982. Behavior and substrate selection during larval settling in the lobster *Homarus americanus*. J. Crustacean Biol. 2: 59–69.

CADDY, J. F. 1979. The influence of variations in the seasonal temperature regime on survival of larval stages of the American lobster (*Homarus americanus*) in the southern gulf of Saint Lawrence. Rapp. P.-v. Réun. Cons. int. Explor. Mer 175: 204–216.

CALINSKI, M. D., AND W. G. LYONS. 1983. Swimming behavior of the puerulus of the spiny lobster *Panulirus argus* (Latreille, 1804) (Crustacea: Palinurdae). J. Crustacean Biol. 3: 329–335.

CARLBERG, J. M., AND J. C. VAN OLST. 1976. Brine shrimp (*Artemia salina*) consumption by the larval stages of the American lobster (*Homarus americanus*) in relation to food density and water temperature, p. 379–389. *In* J. W. Avault Jr. [ed.] Proceedings from the seventh annual meeting (of the) World Mariculture Society, January 25–29, 1976, San Diego, CA. Louisiana State University, Baton Rouge, LA.

COBB, J. S., T. GULBRANSEN, B. F. PHILLIPS, D. WANG, AND M. SYSLO. 1983. Behavior and distribution of larval and early juvenile *Homarus americanus*. Can. J. Fish. Aquat. Sci. 40: 2184–2188.

COBB, J. S., D. WANG, D. B. CAMPBELL, AND P. ROONEY. 1989. Speed and direction of swimming by postlarvae of the American lobster. Trans. Am. Fish. Soc. 118: 82–86.

EAGLES, M. D., D. E. AIKEN, AND S. L. WADDY. 1986. Influence of light and food on larval American lobsters, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 43: 2303–2310.

ENNIS, G. P. 1986a. Swimming ability of larval American lobsters, *Homarus americanus*, in flowing water. Can. J. Fish. Aquat. Sci. 43: 2177–2183.

1986b. Stock definition, recruitment variability, and larval recruitment processes in the American lobster, *Homarus americanus*: a review. Can. J. Fish. Aquat. Sci. 43: 2072–2084.

EPIFANIO, C. E. 1988. Transport of invertebrate larvae between estuaries and the continental shelf. Am. Fish. Soc. Symp. 3: 104–114.

FOGARTY, M. J. 1983. Distribution and relative abundance of American lobster, *Homarus americanus*, larvae: a review, p. 3–8. *In* M. J. Fogarty [ed.] Distribution and relative abundance of American lobster, *Homarus americanus*, larvae: New England investigations during 1974–1979. NOAA Tech. Rep. NMFS SSRF-775.

HADLEY, P. B. 1906. The relation of optical stimuli to rheotaxis in the American lobster, *Homarus americanus*. Am. J. Physiol. 17: 326–343.

HARDING, G. C., J. D. PRINGLE, W. P. VASS, S. J. PEARRE, AND S. J. SMITH. 1987. Vertical distribution and daily movements of larval lobsters *Homarus americanus* over Browns Bank, Nova Scotia. Mar. Ecol. Prog. Ser. 41: 29–41.

HERRICK, F. H. 1909. Natural history of the American lobster. Bull. U.S. Fish. Comm. 15: 149–408.

HUDON, C., AND P. FRADETTE. 1988. Planktonic growth of larval lobster (*Homarus americanus*) off îles de la Madeleine (Quebec), Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 45: 868–878.

HUDON, C., P. FRADETTE, AND P. LEGENDRE. 1986. La répartition horizontale et verticale des larves de homard (*Homarus americanus*) autour des îles de la Madeleine, golfe du Saint-Laurent. Can. J. Fish. Aquat. Sci. 43: 2164–2176.

HUGHES, J. T., R. A. SHLESER, AND G. TCHOBANOGLOUS. 1974. A rearing tank for lobster larvae and other aquatic species. Prog. Fish-Cult. 36: 129–132.

KILS, U. 1982. The swimming behavior, swimming performance and energy balance of Antarctic krill, *Euphausia superba*. Biomass Sci. Ser. 3: 1–121.

PHILLIPS, B. F. 1981. The circulation of the south eastern Indian Ocean and the planktonic life of the western rock lobster. Oceanogr. Mar. Biol. Annu. Rev. 19: 11–39.

PHILLIPS, B. F., AND P. S. MCWILLIAM. 1986. The pelagic phase of spiny lobster development. Can. J. Fish. Aquat. Sci. 43: 2153–2163.

PHILLIPS, B. F., AND L. OLSEN. 1975. Swimming behavior of the puerulus larvae of the Western rock lobster. Aust. J. Mar. Freshwater Res. 26: 415–417.

SASAKI, G. C. 1984. Biochemical changes associated with embryonic and larval development in the American lobster *Homarus americanus* Milne Edwards. Ph.D. thesis. MIT/WHOI, WHOI-84-8.

SASAKI, G. C., J. M. CAPUZZO, AND P. BIESOT. 1986. Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus*. Can. J. Fish. Aquat. Sci. 43: 2311–2319.

SERFLING, S. A., AND R. F. FORD. 1975. Ecological studies of the puerulus larval stage of the California spiny lobster, *Panulirus interruptus*. Fish. Bull. 73: 360–367.

SMITH, S. I. 1873. The early stages of the American lobster (*Homarus americanus* Edwards). Trans. Conn. Acad. Sci. 2: 351–381.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. The principles and practice of statistics in biological research. W. F. Freeman and Co., New York, NY. 413 p.

SULKIN, S. D. 1986. Application of laboratory studies of larval behavior to fisheries problems. Can. J. Fish. Aquat. Sci. 43: 2184–2188.

TEMPLEMAN, W. 1936. The influence of temperature, salinity, light and food conditions on the survival and growth of the larvae of the lobster (*Homarus americanus*) J. Biol. Board Can. 2: 485–497.

VOGEL, S. 1981. Life in moving fluids, the physical biology of flow. Princeton University Press, Princeton, NJ. 352 p.

Interactive Effects of Nutrient Reduction and Herbivory on Biomass, Taxonomic Structure, and P Uptake in Lotic Periphyton Communities[1]

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Steinman, A. D., P. J. Mulholland, and D. B. Kirschtel. 1991. Interactive effects of nutrient reduction and herbivory on biomass, taxonomic structure, and P uptake in lotic periphyton communities. Can. J. Fish. Aquat. Sci. 48: 1951–1959.

Four treatments were imposed on eight laboratory streams in a factorial design to examine the roles of nutrient reduction and herbivory on periphyton communities. Treatments included two flow regimes (once-through flow or 90% recirculated water) and two levels of grazer density (1000 or 0·m^{-2}, using the snail *Elimia clavaeformis*). Periphyton biomass was significantly greater in streams without snails than in those with them, but water supply had no overall significant effect on biomass, even though inorganic P and N concentrations were significantly lower in recirculated than in once-through streams. Areal-specific P uptake rates (measured with ^{33}P) were significantly greater on two dates in no-snail streams compared with snail streams, presumably because of the greater biomass levels in the former systems. Differences in biomass-specific P uptake rates were not significantly affected by either grazer density or water supply. Relative abundances of most algal species were unaffected by the water supply treatment, although percent biovolume of two *Epithemia* species was greater in no-snail, recirculated than in no-snail, once-through streams. Grazing activity dramatically reduced the percent biovolume of species with upright growth forms, resulting in dominance by species with prostrate growth forms.

Quatre traitements ont été appliqués à huit cours d'eau en laboratoire selon un plan d'expérience factoriel afin d'étudier le rôle de la réduction des éléments nutritifs et des herbivores sur des communautés de périphyton. Les traitements comprenaient deux régimes de débit (circulation unique ou recirculation de l'eau à 90 %) et deux niveaux de densité des brouteurs (1 000 ou 0·m^{-2}; organisme utilisé : *Elimia clavaeformis*). La biomasse du périphyton était significativement supérieure dans les cours d'eau dépourvus d'escargots à ce qu'elle était dans les autres, mais, dans l'ensemble, l'alimentation en eau n'avait aucun effet significatif sur la biomasse même si la concentration en P et N inorganique était significativement moindre dans l'eau recirculée que dans l'eau non recirculée. Le taux d'absorption de P par unité de surface (mesuré au moyen du ^{33}P) a été significativement supérieur à deux dates dans les cours d'eau sans escargot à comparer aux cours d'eau avec escargots; on peut penser que cela est attribuable à la plus grande biomasse trouvée dans les premiers circuits. Les différences dans le taux d'absorption du P en fonction de la biomasse n'ont pas été significativement modifiées par la densité des brouteurs ni le mode d'alimentation en eau. L'abondance relative de la plupart des espèces algaires n'a pas été modifiée par le mode d'alimentation en eau même si le volume de matière organique, en pourcentage, de deux espèces d'*Epithemia* était supérieur dans les cours d'eau recirculée et sans escargot que dans les cours d'eau sans recirculation et sans escargot. L'activité des brouteurs réduisait considérablement le volume de matières organiques, en pourcentage, des espèces qui avaient un port dressé; cela se traduisait par une dominance des espèces au port plat.

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The abundance, productivity, and structure of periphyton communities can be strongly influenced both by nutrient concentration and herbivory. Addition of nutrients to nutrient-limited streams has resulted in increased periphyton biomass and productivity, as well as distinct changes in algal community structure (Stockner and Shortreed 1978; Elwood et al. 1981; Peterson et al. 1983; Grimm and Fisher 1986; Pringle 1987, 1990; Hill and Knight 1988; Bothwell 1989; Winterbourn 1990). Moderate to high herbivore activity usually reduces periphyton biomass, increases biomass-specific productivity but decreases areal productivity, and leads to dominance by prostrate growth forms in the periphyton community (review by Gregory 1983; see also Lamberti and Resh 1983; Jacoby 1985; Hill and Knight 1987; Steinman et al. 1987; Lamberti et al. 1989).

The present study examines the influence of nutrients and herbivory on periphyton communities, but differs from the above studies in two ways: (1) instead of examining the influence of nutrient addition on periphyton communities, our experimental design allows us to test the effect of nutrient reduction on the periphyton; and (2) we examine the interactive effects of nutrients and herbivory, instead of focusing on just one of these factors. Although a few studies have examined the

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interactive effects of nutrients and herbivory on periphyton in streams (Summer and McIntire 1982; Stewart 1987; McCormick and Stevenson 1989), these studies used nutrient enrichments. We chose to examine nutrient reduction, rather than enrichment, because (1) we were interested in determining if nutrient recycling could be enhanced if external nutrient supplies were reduced, (2) we wanted to apply our results to natural spring-fed streams in this region and elsewhere, where longitudinal reductions in nutrient concentrations occur (P. J. Mulholland, unpubl. data), and (3) we anticipated that the potentially beneficial process of nutrient excretion by herbivores (Cuker 1983; Sterner 1986) would be more detectable in an experimental design such as ours, where nutrient limitation instead of saturation was the desired goal.

In the present study, we report the response of periphyton biomass, nutrient uptake dynamics, and algal community structure to the nutrient/herbivore manipulations. Mulholland et al. (1991) focused primarily on the ecosystem responses (i.e. production, nutrient cycling) to these manipulations.

Materials and Methods

Experimental Design

Experiments were conducted in laboratory stream channels described by Steinman et al. (1990). Briefly, we used eight U-shaped, fiberglass streams, each 0.3 m wide and 22 m long. Water was supplied by a spring-fed pond at 0.64 $L \cdot s^{-1}$, resulting in a current velocity of 22 $cm \cdot s^{-1}$. In-line sand filters removed most incoming particulate matter. Irradiance was supplied by 400-W metal halide lamps that provided approximately 150 $\mu mol\ quanta \cdot m^{-2} \cdot s^{-1}$ when measured with a Li-Cor Li-188B quantum meter at the water surface at midchannel. Daily photoperiod was set at 8 h light:16 h dark.

Unglazed ceramic cylinders (1.6 × 1.6 cm) were placed at the bottom of each stream (18 000 per stream) and served as substrates for periphyton colonization and units for sampling. To inoculate the streams, rocks were collected from a second-order reach of Walker Branch, a stream on the U.S. Department of Energy's Oak Ridge reservation; they were placed in the streams in September 1987.

Beginning in March 1988, four treatments were imposed on the eight channels, giving two replicate streams per treatment: (1) snails, recirculated flow; (2) snails, once-through flow; (3) no snails, recirculated flow; and (4) no snails, once-through flow. In the once-through streams, all incoming water left the channel after making a 22-m circuit. In the recirculated streams, 90% of the water was recirculated back to the upstream end after making a 22-m circuit, resulting in a continuous mix of 10% new water and 90% recirculated water. Each stream had its own independent heat exchange unit (Steinman et al. 1990) so that water temperatures in the recirculated streams were regulated to within 1°C of the temperature in the once-through streams.

The snail treatment consisted of adding the prosobranch snail *Elimia clavaeformis* Lea to half of the once-through and half of the recirculated streams at a density of $1000 \cdot m^{-2}$, a density commonly found in streams on the Oak Ridge reservation (Burris et al. 1990). Although the other streams contained no *Elimia*, chironomid larvae and microfauna were present, so even the streams without snails were exposed to a low level of grazing. The experiment lasted 120 d, with parameters measured at either weekly or monthly intervals.

Sampling Design and Analytical Methods

Samples for water chemistry were collected weekly at the upstream end of each stream. Samples were filtered through stream-water-washed glass fiber filters (Gelman A/E, pore size 1 μm). In this paper, only the N and P data are reported. Concentrations of NH_4-H were measured by phenate colorimetry using a Technicon TRAACS 800 autoanalyzer and concentrations of $NO_3 + NO_2$-N by Cu–Cd reduction followed by automated colorimetric analysis (USEPA 1983). Soluble reactive phosphorus (SRP) was measured using the ascorbic acid method (APHA 1985).

Periphyton biomass was measured as ash-free dry mass (AFDM). Ten cylinders were selected randomly (five upstream and five downstream) from each stream 3 d prior to and 22, 50, 85, and 113 d after the initiation of the treatments. Cylinders were dried for 24 h at 60°C, weighed to the nearest 0.01 mg, combusted for 12–14 h at 475°C, and reweighed. AFDM was calculated as the difference in mass between the dried and combusted samples.

Rates of P uptake from water by periphyton were determined by measuring short-term uptake of ^{33}P. Eight cylinders were selected randomly from each stream (four upstream and four downstream) and incubated with 1 L of filtered stream water (from their stream of origin) in stirred glass chambers (50 rpm) at ambient stream temperatures. Approximately 18.5 kBq of carrier-free $^{33}PO_4$ was added to each chamber. One-millilitre subsamples of water were removed at 5, 10, 15, 30, 45, and 60 min, filtered, and assayed for the radiotracer by liquid scintillation. Adsorption of isotope to glass walls was <2% of uptake, so no corrections were made for adsorption. Concentrations of SRP in the water of each stream were measured to compute total P uptake from the first-order rate coefficient of radiotracer depletion in the water (assuming all SRP was as biologically available PO_4). Total P uptake rates were measured on days 21, 56, 85, and 112 of the experiment and calculated both per unit area and AFDM of periphyton.

For the quantitative analysis of algal community structure, eight cylinders (four upstream and four downstream) were selected randomly from each stream 3 d prior to and 23, 58, 87, and 115 d following the initiation of treatments. Attached algae were removed from each cylinder with a toothbrush and pooled into an upstream and a downstream sample for each stream. The resulting slurries were homogenized with a teflon tissue grinder to disrupt clumps. Slides for microscopic examination were prepared using the procedures described by Stevenson (1984). At least 500 cells per slide were examined at 1000× magnification and only cells with intact chloroplasts were counted. Cell counts for each species were converted to biovolume using standard geometric formulae. The effects of treatments on algal physiognomy and growth form were assessed by assigning each of the dominant algal species, a posteriori, to one of five growth forms (prostrate, filamentous, upright nonfilamentous, stalked with endosymbionts, and colonial).

Statistical Analysis

A two-way repeated measures analysis of variance (ANOVA) was used to analyze data, with the main effects being presence/absence of snails and the type of water supply (i.e. recirculation or once-through). If there was a significant interaction between time and treatment effect or between time and treatment interaction, data were analyzed by individual sampling dates. Other-

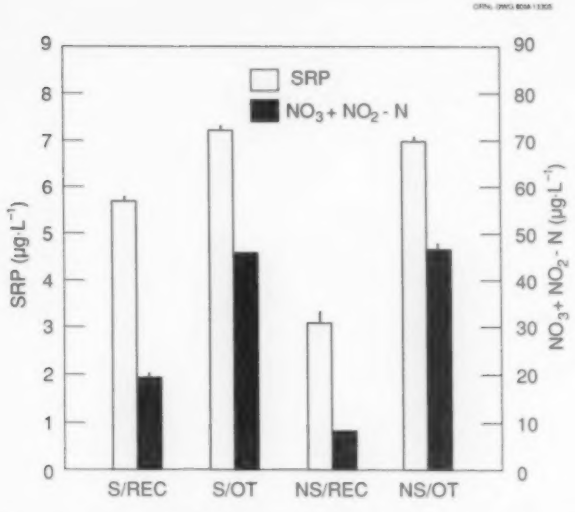

FIG. 1. Mean dissolved inorganic concentrations of soluble reactive phosphorus (SRP) and $NO_2 + NO_3$ in incoming water for the different treatments pooled over the entire experimental period ($n = 13$). Error bar = 1 SE. S, snails; NS, no snails; OT, once-through water supply; REC, 90% recirculated water supply.

wise, data were analyzed by the overall repeated measures ANOVA (Morrison 1967). If necessary, AFDM and P uptake data were log transformed and algal community structure data were arcsine-square-root transformed prior to analysis to correct for heterogeneity of variances.

Results

The water supply and snail treatments resulted in significant effects on inorganic P and N concentrations (Fig. 1; Table 1). Both SRP and $NO_2 + NO_3$ concentrations were significantly higher in once-through than in recirculated streams. SRP and $NO_2 + NO_3$ concentrations were also significantly higher in snail than in no-snail streams, but only within recirculated treatments. Thus, the snail × water supply interaction term was significant (Table 1). Concentrations of NH_4 were less than the detection limit (2 μg $N \cdot L^{-1}$) throughout the study.

Prior to the initiation of the treatments, mean periphyton biomass was somewhat higher in the streams that were to become no snail, recirculated relative to the other streams, but this difference was not significant (Fig. 2). The relatively low AFDM in no-snail, once-through streams was attributable to a large sloughing event in one of the two replicate streams that occurred just prior to the samples being taken. After treatments began, AFDM was significantly reduced in streams with snails (Fig. 2; Table 1). Although water supply had no overall significant effect on AFDM, a significant interaction term resulted because AFDM was higher in recirculated than in once-through streams, but only in the no-snail treatments (Fig. 2; Table 1). A sloughing event on day 46 resulted in biomass declines in the no-snail streams. However, biomass began to accrue soon after and continued to increase in the no-snail streams until the end of the experiment (Fig. 2).

In addition to analysis of AFDM as absolute values, biomass data also were analyzed as percent changes from the pretreatment values. In this case, AFDM again was significantly lower in streams with snails compared with those without them. Mean increases in biomass were greater in no-snail, once-through streams than in no-snail, recirculated streams (consistent with analysis using absolute AFDM data). However, this difference was no longer statistically significant because there was high variance associated with relative biomass changes in no-snail, once-through streams (because one of the replicate streams experienced a large sloughing event prior to the initial measurements, thereby resulting in low initial values and subsequent large percent changes compared with its replicate: percent increases in AFDM ranged from 2 to 35% in one replicate and from 210 to 570% in the replicate with initial sloughing).

Areal-specific P uptake rates were significantly greater in streams without snails than in those with them on days 21 and 112 (Fig. 3; Table 1). Water supply had no significant influence on areal P uptake on any date. Mean biomass-specific P uptake rates by periphyton were greater in snail, once-through streams than in no-snail, recirculated streams, respectively, on each date, but these differences were not statistically significant ($0.10 > P > 0.05$) (Fig. 3; Table 1). No significant interactions with time were detected in the repeated measures ANOVA (Table 1), indicating that biomass-specific P uptake values showed no distinct temporal pattern in any of the streams throughout the study.

Prior to the start of the experimental treatments, the diatom *Achnanthes minutissima* comprised approximately 15% of algal community biovolume in streams that were to lack snails, compared with <5% in streams that were to have snails (Fig. 4). After treatments began, relative biovolume of *A. minutissima* was significantly greater in no-snail than in snail streams and marginally greater in once-through than in recirculated streams (Fig. 4; Table 1). The red alga *Batrachospermum moniliforme* comprised between 20 and 40% of algal community biovolume in all streams before the treatments were started (Fig. 4). Once treatments had begun, *Batrachospermum* relative biovolume was significantly greater in snail than in no-snail streams, and it often accounted for >50% of community biovolume in snail streams. Although erect filaments were occasionally observed, the "chantransia" stage (i.e. a crust of mostly prostrate filaments) often bearing monosporangia was the predominant growth form of *Batrachospermum* in the streams with snails.

Stigeoclonium is a heterotrichous alga capable of growing as an erect filament or as a mass of prostrate basal cells. Before the start of the treatments, *Stigeoclonium* was observed primarily in the erect form and comprised about 10% of algal community biovolume in all treatments (Fig. 4). Once treatments began, *Stigeoclonium* percent biovolume was significantly greater in snail than in no-snail streams, although water supply had no significant effect (Fig. 4; Table 1). Algal growth form was exclusively prostrate basal cells or very short filaments (<10 cells long) in the streams with snails whereas both short and long filaments were observed in streams without snails.

The filamentous cyanobacterium *Oscillatoria* was not observed in any of the streams until day 87 of the experiment (Fig. 4). Very little *Oscillatoria* was observed in snail, recirculated streams on day 87 or 115. Its relative abundance was variable in the other streams, with treatments having no significant influence on its percent biovolume (Table 1).

The diatom *Synedra ulna* usually grows in clusters of upright rosettes. It was not detected before treatments were started, but its percent biovolume was significantly greater in snail than in no-snail streams on days 23 and 58 after treatments began

TABLE 1. Repeated measures ANOVA results for inorganic nutrient concentration in the incoming stream water, AFDM, rates of P uptake, and algal taxonomic structure. Data presented are F-values (*$P < 0.10$; **$P < 0.05$; ***$P < 0.01$). For parameters where there was a significant treatment × time interaction ($P < 0.05$), ANOVA results are given on each sampling date.

Parameter	Date[a]	Treatment effect		
		Snails	Water supply	Snails × water
Nutrients (water)				
SRP[b]	All (4)	34.23***	129.85***	24.23***
NO_3-N	All (4)	52.85***	2177.11***	75.40***
AFDM[c]	All (4)	183.04***	0.22	16.21**
Biomass-specific P uptake	All (4)	6.17*	5.22*	0.05
Areal-specific P uptake	Day 21	35.31**	2.38	2.50
	Day 56	0.02	1.37	2.24
	Day 85	3.72	1.44	1.59
	Day 112	13.68**	4.24	2.93
Taxonomic structure				
Achnanthes minutissima	All (4)	14.43**	4.59*	3.37
Batrachospermum moniliforme	All (4)	36.27***	0.36	0.01
Epithemia adnata	All (2)	80.93***	2.61	2.61
Epithemia turgida	All (3)	111.82***	1.45	12.11**
Fragilaria capucina	Day 23	16.18**	<0.01	0.73
	Day 58	407.66***	0.10	0.10
	Day 87	2.78	0.02	0.46
	Day 115	1.62	0.38	0.38
Oscillatoria sp.	Day 87	0.29	5.08*	0.18
	Day 115	4.69*	<0.01	0.65
Stigeoclonium sp.	All (4)	10.57**	0.06	1.68
Synedra ulna	All (2)	33.46***	0.05	0.43

[a]Date on which measurements were made. "All" refers to use of all sampling dates in repeated measures ANOVA with total number of dates used in parentheses.
[b]SRP = soluble reactive phosphorus.
[c]AFDM = ash-free dry mass (of periphyton).

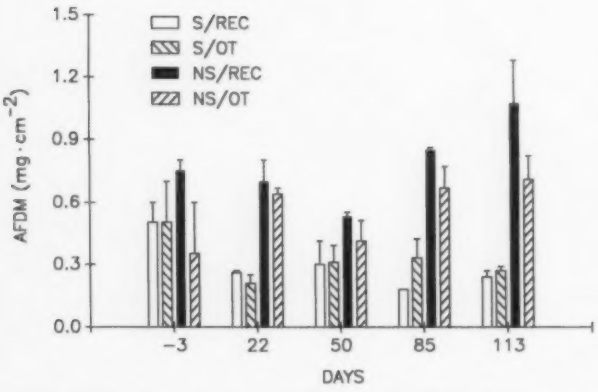

FIG. 2. Mean ash-free dry mass (AFDM) of periphyton for each treatment over time. "Day −3" data indicate values were obtained before treatments were initiated. Error bars = 1 SE. See Fig. 1 for treatment definitions.

(Fig. 5; Table 1). The chain-forming diatom *Fragilaria capucina* comprised substantial and very similar percent biovolumes in all streams before the treatments began (Fig. 5). On days 23 and 58 after treatments were initiated, relative biovolume of *Fragilaria* was significantly greater in no-snail than in snail streams, with water supply having no effect (Fig. 5; Table 1). Percent biovolume of *Fragilaria* declined over the course of the experiment, and although both relative and absolute biovolumes remained greater in no-snail than in snail streams on days 87 and 115, these differences were not statistically significant (Fig. 5; Table 1).

Epithemia spp. can attach to substrates adnately or by mucilaginous stalks and are capable of harboring endosymbiotic cyanobacteria (Geitler 1977). Both *Epithemia* species in this study were observed only after day 58 of the experiment (Fig. 5). When present, *E. adnata*'s relative biovolume was significantly greater in no-snail than in snail streams, and although mean percent biovolume was greater in no-snail, recirculated than in no-snail, once-through streams, this difference was not significant (Fig. 5; Table 1). *Epithemia turgida* also comprised a significantly greater percent biovolume of communities in no-snail than in snail streams. Percent biovolume of this taxon was significantly greater in recirculated than in once-through streams, but only in no-snail treatments, which accounts for the significant interaction term in the ANOVA (Table 1).

Grazing had a profound effect on algal physiognomy. Upright nonfilamentous, filamentous, and stalked forms with endosymbionts dominated the no-snail streams (Fig. 6). Water supply appeared to have relatively little effect on algal physiognomy, although the endosymbiont-containing stalked forms appeared to be more prevalent in no-snail, recirculated than in no-snail, once-through streams.

Discussion

Additions of nutrients to stream systems usually result in increases in periphyton biomass (Elwood et al. 1981; Peterson

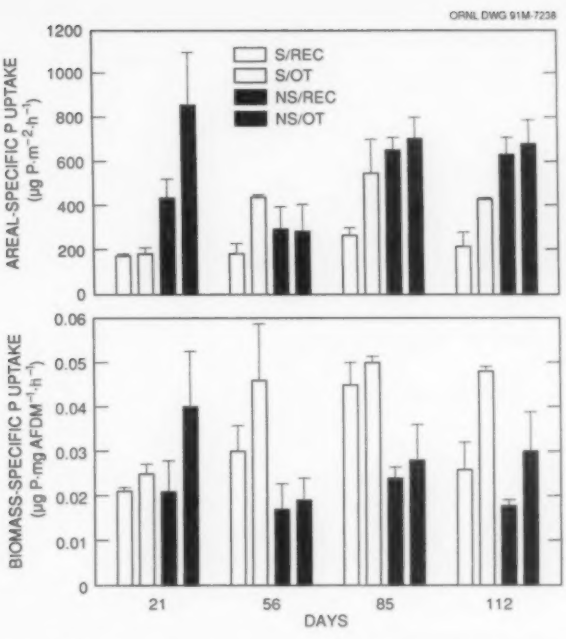

FIG. 3. Mean areal-specific (top panel) and biomass-specific (bottom panel) uptake rates of P for each treatment over time. Error bars = 1 SE. See Fig. 1 for treatment definitions.

et al. 1985; Pringle 1987; Bothwell 1989) whereas additions of herbivores usually result in declines in periphyton biomass (Lamberti and Resh 1983; Jacoby 1985; Steinman et al. 1987). However, if periphyton growth is limited by a resource other than nutrients (e.g. light), biomass accumulation may not be stimulated by nutrient addition (Gregory 1980; Lowe et al. 1986; Hill and Knight 1988). Similarly, herbivore additions need not result in biomass declines if herbivores are vulnerable to predation from a higher trophic level (Allan 1983; Power et al. 1985), dominant components of the periphyton are already resistant to herbivory (Moore 1975; Steinman et al. 1987), or the streams have unique environmental properties, such as in thermal springs (e.g. Stockner 1971; Collins et al. 1976).

In the present study, we investigated the influence of nutrients by reducing instead of increasing their concentration. Our experimental design was successful in that nutrient concentrations declined significantly in recirculated compared with once-through streams. We attribute this reduction to (1) the lower input of new water into recirculated streams and (2) the longer residence time of water in recirculated streams, which in turn allowed the biota greater opportunity to deplete nutrients in the water. We anticipated, in turn, that these reduced nutrient concentrations in recirculated steams would translate into lower biomass levels compared with once-through streams.

However, nutrient reduction did not result in lower biomass levels. Indeed, mean AFDM in no-snail, recirculated streams actually was higher than in no-snail, once-through streams on all sampling dates. Two possible explanations may account for nutrient reduction not resulting in lower periphyton biomass in no-snail, recirculated than in no-snail, once-through streams: (1) a resource other than some nutrient was limiting biomass accrual in once-through streams and (2) nutrient recycling rates became enhanced in recirculated streams. Both explanations seem plausible. In the case of explanation (1), irradiance levels may have limited periphyton biomass once the mats became well developed. Jasper and Bothwell (1986) reported that suspended periphyton communities exhibited optimal photosynthetic rates at irradiance levels that exceeded 400 μmol quanta·m^{-2}·s^{-1}. However, the irradiance levels at the stream surface in our study (150 μmol quanta·m^{-2}·s^{-1}) approach or exceed I_k levels for periphyton communities in Quebec (Paul and Duthie 1989) and eastern Tennessee (Boston and Hill 1991). Thus, it is likely that periphyton communities in our study were fixing C at high, albeit not maximal, rates. With respect to explanation (2), no-snail, recirculated streams showed greater indices of nutrient recycling than no-snail, once-through streams (Mulholland et al. 1991), which no doubt ameliorated some of the effects of nutrient reduction. In particular, higher ratios of total to net P uptake and higher levels of ATP-specific phosphatase activity were measured in no-snail, recirculated than in no-snail, once-through streams (Mulholland et al. 1991).

Heavy grazing pressure by *Elimia* limited periphyton biomass accrual and accounted for the absence of significant AFDM differences between snail, recirculated and snail, once-through streams. As a consequence of nutrient regeneration by herbivores (Mulholland et al. 1991; see also Cuker 1983; Sterner 1986) and the low periphyton biomass in snail streams, differences in nutrient concentration between recirculated and once-through streams were smaller in streams with snails than those without them. Thus, nutrient concentration was less likely to be limiting in the thin, sparse communities present in streams with snails.

The greater biomass-specific P uptake rates from water by periphyton in snail than in no-snail and in once-through than in recirculated streams reflect greater P demand by periphyton in snail and once-through assemblages. In the case of grazed versus ungrazed assemblages, other studies have shown that grazing can increase the biomass-specific productivity of periphyton (Lamberti and Resh 1983; Stewart 1987; Lamberti et al. 1989), which in turn (because of stoichiometric needs) may result in higher uptake rates of nutrients. In the present study, however, biomass-specific ^{14}C fixation rates were not statistically different between snail and no-snail streams (Mulholland et al. 1991). We believe that biomass-specific P uptake was greater in grazed than in ungrazed periphyton because lower periphyton biomass in snail streams allowed more of the nutrient demand to be met by the nutrient supply in the overlying water than by nutrient recycling within the periphyton mat. Although biomass-specific rates of P uptake were higher in snail than in no-snail streams, areal-specific P uptake was significantly greater in streams without snails compared with streams with snails (on two of four dates) simply because there was much more biomass present in streams without snails than in streams with them. This is consistent with the findings reported in Mulholland et al. (1983), where grazers increased the spiralling length of P (i.e. P was less tightly cycled) because the reduction of periphyton biomass by grazers influenced spiralling length more (by resulting in less overall P uptake) than the increases in biomass-specific metabolism associated with grazing actvity.

The greater P uptake rates in once-through than in recirculated streams can be attributed to periphyton in once-through streams obtaining more of their P from the water column than from recycling. The demand for P in recirculated streams was met by greater reliance on recycling (Mulholland et al. 1991),

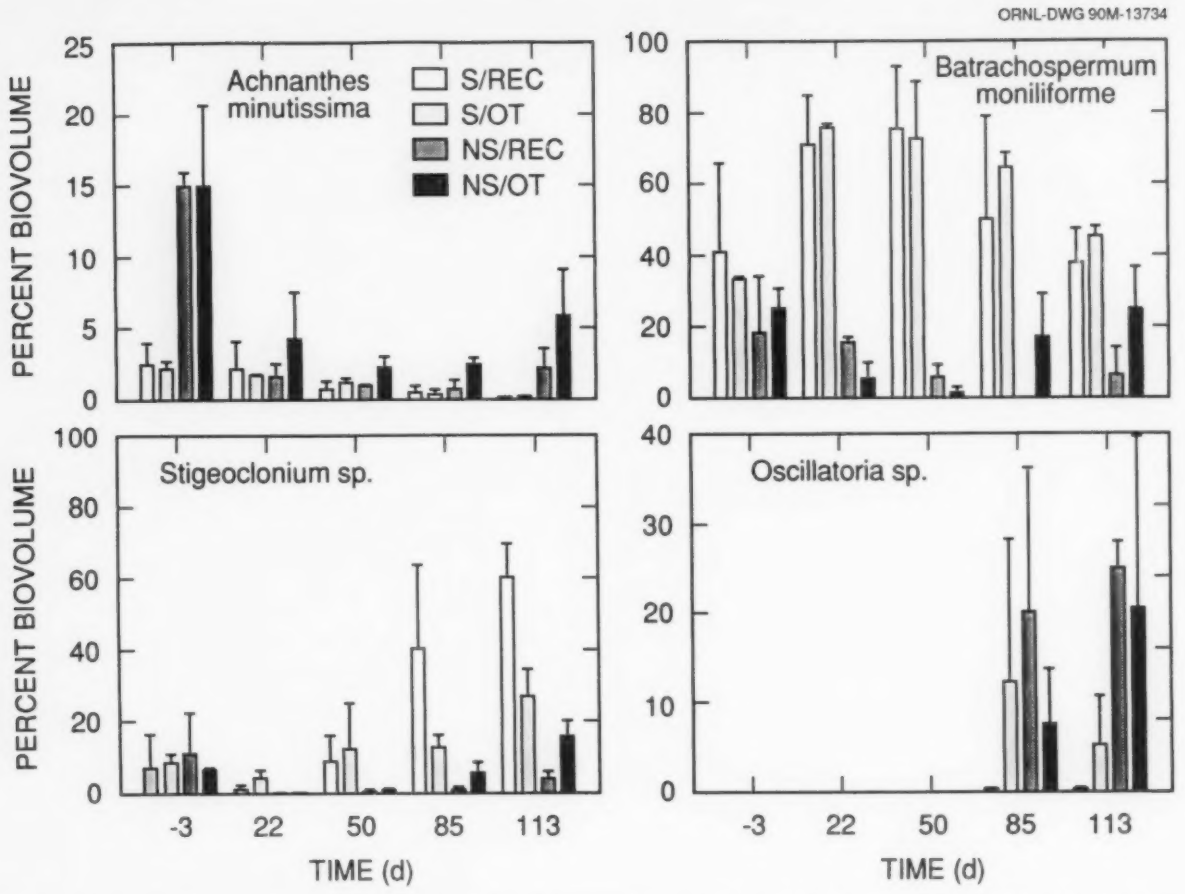

FIG. 4. Mean percent biovolume of dominant algal taxa for each treatment over time. "Day −3" data indicate values were obtained before treatments were initiated. *Oscillatoria* was not observed on the first three sampling dates. Error bar = 1 SE. See Fig. 1 for treatment definitions.

and proportionately less of the P demand was met by the external water supply. Mazumder et al. (1989) and Paul and Duthie (1989) also noted that periphyton communities with high P demand may have high recycling of assimilated P. Algal growth form can influence nutrient uptake rates. Odum et al. (1958) reported that macroalgae with a high surface area to volume ratio had greater biomass-specific P uptake rates than forms with a low surface area to volume ratio. In our study, species with a filamentous growth form were in greater abundance in no-snail, once-through than in no-snail, recirculated streams (repeated measures ANOVA; $P < 0.05$). This growth form not only has a relatively high surface area to volume ratio compared with other periphytic growth forms (unpubl. data), but its streaming nature allows it to project beyond the boundary layer surrounding the periphyton matrix, where diffusion might limit nutrient mass transfer into the mat (Riber and Wetzel 1987). Consequently, a greater abundance of filamentous species may have contributed to the greater biomass-specific P uptake rates in no-snail, once-through compared with no-snail, recirculated streams.

Despite the change in filamentous growth form abundance, changes in the abundance of individual algal species were relatively insensitive to water supply. Only *A. minutissima*, *Oscillatoria* sp., and *E. turgida* showed even marginally significant effects to nutrient level. Percent biovolume of *A. minutissima* was greater in once-through than in recirculated streams. Other studies, using nutrient-diffusing substrates and enrichment of the water, also have shown than *A. minutissima* responds positively, albeit not always significantly, to nutrient enrichment (Fairchild and Lowe 1984; Lowe et al. 1986; Carrick et al. 1988; Hill and Knight 1988; Pringle 1990). *Achnanthes minutissima* also may be a dominant component of algal communities in unenriched environments. This taxon may be well adapted to exploit low-nutrient environments because of their relatively high surface area to volume ratio and vertical growth habit (i.e. attachment by mucilage pad; Pringle 1990).

Both *Epithemia* species exhibited greater percent biovolumes in no-snail, recirculated than in no-snail, once-through streams. Many species in the Epithemiaceae harbor endosymbiotic cyanobacteria, which are capable of fixing N_2 (Drum and Pankratz 1965; Geitler 1977). These species would be at a competitive advantage for N in recirculated streams if their endosymbionts actively fixed N_2. Hill and Knight (1988) reported greater biovolumes of *E. turgida* and *Rhopalodia gibba* on control substrates than on those releasing either P, N, or N + P. Fairchild and Lowe (1984) also reported that biovolumes of *E. adnata* and *R. gibba* were not affected by additions of N, although their biovolumes increased on substrates releasing P.

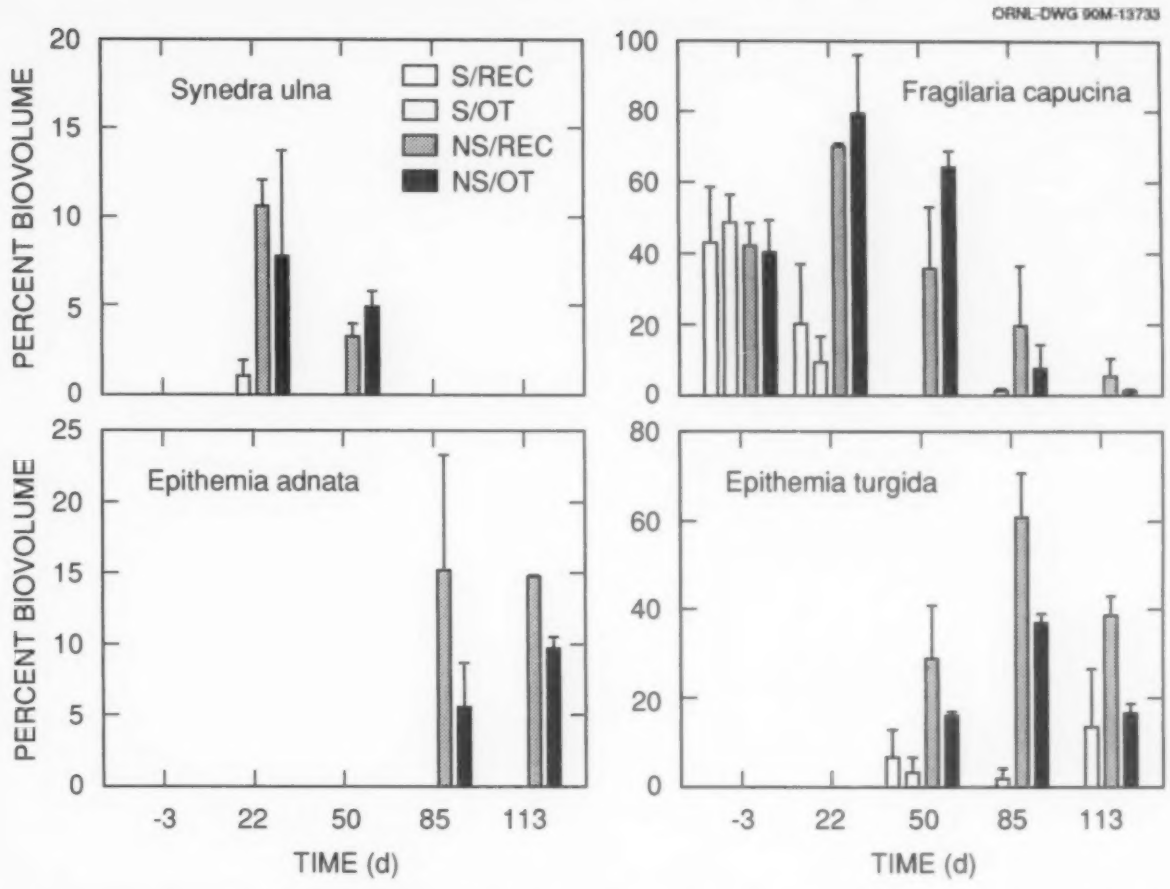

FIG. 5. Mean percent biovolume of dominant algal taxa for each treatment over time. "Day −3" data indicate values were obtained before treatments were initiated. *Synedra ulna* was not observed on the first, fourth, or fifth sampling dates, *E. turgida* was not observed on the first two sampling dates, and *E. adnata* was not observed on the first three sampling dates. Error bar = 1 SE. See Fig. 1 for treatment definitions.

This is consistent with the idea that N_2-fixing endosymbionts in members of the Epithemiaceae confer a competitive advantage to their hosts as N:P ratios decline in the water. The inorganic N:P ratio in stream water from the present study was about 50% lower in recirculated streams (6–8:1) than in once-through streams (15:1). Thus, the greater abundance of *Epithemia* in no-snail, recirculated streams may have been a competitive response to the changing nutrient ratios. Although N_2-fixation assays were not conducted in the present study, microscopic analyses of both *Epithemia* species revealed the presence of endosymbionts. The absence of free-living N_2-fixing cyanobacteria in recirculated streams may have been due to (1) their absence in the species pool or (2) P limitation. Because no samples were taken from the rocks used to inoculate our laboratory streams, it is impossible to evaluate the former possibility. However, *Nostoc* communities have been observed in the stream from which the rocks were collected, so it is possible that N_2-fixers were present in the original species pool. Although the abundance of cyanobacteria in lentic systems has been shown to be dependent on low N:P availability in water (Schindler 1971; Stockner and Shortreed 1988), it also has been reported that relatively high P concentrations are required for them to become abundant (Liao 1977; Stockner and Shortreed 1988). Hence, it is possible that free-living N_2-fixing cyanobacteria were present in the original species pool, but became competitively excluded because of the low P concentrations in the water.

The taxonomic structure of the periphyton changed very little in response to our water supply treatment. This contrasts with results from enrichment studies using nutrient-diffusing substrates (e.g. Carrick and Lowe 1988; Pringle 1990) and is attributable, at least in part, to the fact that periphyton communities in our study were exposed to small changes in absolute nutrient concentration. Yet, this absolute change is one of ecologically realistic levels for streams of this region (Elwood and Turner 1989). Thus, these communities appear relatively resistant to small-scale reductions in nutrient concentration. Pringle (1990) also observed little change in taxonomic structure when inorganic NO_3 and PO_4 were added to the water column (as opposed to the use of diffusing substrates) in relatively small amounts (i.e. from 40 to 80 μg NO_3-N·L^{-1} and from 2.5 to 12.5 μg PO_4-P·L^{-1}. The apparent resistance of algal taxonomic structure may stem either from metabolic flexibility in nutrient uptake rates and cell quotas at the individual/popula-

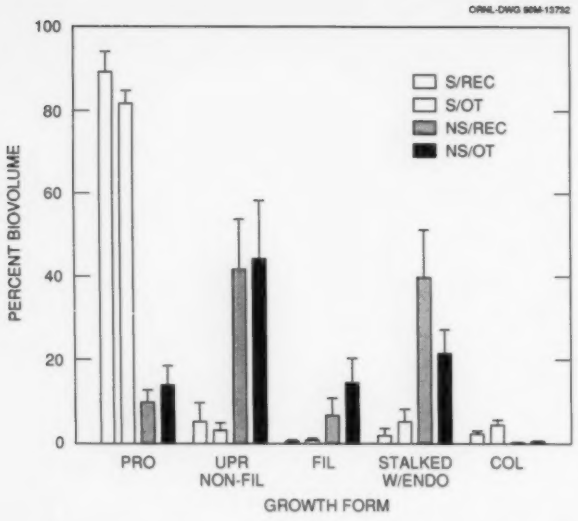

FIG. 6. Mean percent biovolumes of dominant and subdominant algal taxa classified according to growth form for each treatment pooled over the entire experimental period. Prostrate (PRO) species include *A. minutissima*, *B. moniliforme* (chantransia), *Cocconeis placentula*, *Stigeoclonium* basal cells, and *Navicula seminulum*. Upright nonfilamentous (UPR NON-FIL) species include *F. capucina*, *Synedra capitata*, *S. ulna*, and *Amphipleura pellucida*. Filamentous (FIL) species include *Stigeoclonium* (erect), *Oscillatoria* sp., and an unidentified cyanobacterium (No. 1). Stalked species with endosymbionts (STALKED W/ENDO) include *E. adnata* and *E. turgida*. Colonial (COL) species include two unidentified cyanobacteria (Nos. 3 and 4). Error bar = 1 SE. See Fig. 1 for treatment definitions.

tion level or from community/ecosystem-level dynamics that allow higher nutrient recycling rates to buffer the communities from taxonomic change as nutrient concentrations decline. However, we suspect that the taxonomic structure and the types of growth forms present in the periphyton communities would have exhibited greater changes if nutrient concentrations were altered to a greater degree in our experimental treatments.

Herbivory had a much more dramatic effect on algal taxonomic structure than water supply. Percent biovolumes of all dominant algal species were significantly influenced by grazing at some point during the experiment (Table 1). As summarized in Fig. 6, the influence of grazing on community structure was a direct function of algal growth form. Consistent with results from other studies, grazing resulted in a greater relative abundance of prostrate species whereas ungrazed communities were dominated by species that grow or attach both in an upright (e.g. rosette, filamentous, or stalked; see Gregory 1983; Hill and Knight 1987; Steinman et al. 1987, 1989; Lowe and Hunter 1988; Lamberti et al. 1989) and a prostrate manner. Indeed, plant growth form has been shown to be a critical factor in plant–herbivore interactions not only for periphyton, but also for marine macroalgae (Lubchenco and Cubit 1980; Steneck and Watling 1982), phytoplankton (Porter 1973), and terrestrial vegetation (Abrahamson 1975; Crawley 1983).

The results from the present study have implications for periphyton distribution patterns in streams where grazer densities are high and nutrient levels are low. Based on data from our study, we predict that herbivory can be the primary factor limiting periphyton biomass. Elevated irradiance levels or nutrient concentrations may allow the productive capacity of periphyton to exceed consumption demand of grazers in the short run (Elwood et al. 1981; Lamberti et al. 1989), but increased grazer recruitment should ultimately allow the herbivores to once again attain the upper hand in the interaction (cf. Stewart 1987). The amount of time required will be a function of grazer growth rate and fecundity, predation rates from higher trophic levels, and the spatiotemporal scale of manipulation that stimulated periphyton growth to begin with. Long-term field experiments are needed to test this prediction.

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References

ABRAHAMSON, W. G. 1975. Reproductive strategies in dewberries. Ecology 56: 721–726.

ALLAN, J. D. 1983. Predator–prey relationships in streams, p. 191–230. *In* J. R. Barnes and G. W. Minshall [ed.] Stream ecology: application and testing of general ecological theory. Plenum Press, New York, NY.

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). 1985. Standard methods for the examination of water and waste water. 16th ed. American Public Health Association, Washington, DC.

BOSTON, H. L., AND W. R. HILL. 1991. Photosynthesis–light relations of stream periphyton communities. Limnol. Oceanogr. 36: 644–656.

BOTHWELL, M. L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. Can. J. Fish. Aquat. Sci. 46: 1293–1301.

BURRIS, J. A., M. S. BAMFORD, AND A. J. STEWART. 1990. Behavioral responses of marked snails as indicators of water quality. Environ. Toxicol. Chem. 9: 69–76.

CARRICK, H. J., AND R. L. LOWE. 1988. Response of Lake Michigan benthic algae to in situ enrichment with Si, N, and P. Can. J. Fish. Aquat. Sci. 45: 271–279.

CARRICK, H. J., R. L. LOWE, AND J. T. ROTENBERRY. 1988. Guilds of benthic algae along nutrient gradients: relationships to algal community diversity. J. N. Am. Benthol. Soc. 7: 117–128.

COLLINS, N. C., R. MITCHELL, AND R. G. WIEGERT. 1976. Functional analysis of a thermal spring ecosystem, with an evaluation of the role of consumers. Ecology 57: 1221–1232.

CRAWLEY, M. J. 1983. Herbivory. The dynamics of animal–plant interactions. University of California Press, Berkeley, CA. 437 p.

CUKER, B. E. 1983. Grazing and nutrient interaction in controlling the activity and composition of the epilithic algal community of an arctic lake. Limnol. Oceanogr. 28: 133–141.

DRUM, R. W., AND S. PANKRATZ. 1965. Fine structure of an unusual cytoplasmic inclusion in the diatom *Rhopalodia*. Protoplasma 60: 141–149.

ELWOOD, J. W., J. D. NEWBOLD, A. F. TRIMBLE, AND R. W. STARK. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62: 146–158.

ELWOOD, J. W., AND R. R. TURNER. 1989. Streams: water chemistry and ecology, p. 301–350. *In* D. W. Johnson and R. I. Van Hook [ed.] Analysis of biogeochemical cycling in Walker Branch Watershed. Springer-Verlag, New York, NY.

FAIRCHILD, G. W., AND R. L. LOWE. 1984. Artificial substrates which release nutrients: effects on periphyton and invertebrate succession. Hydrobiologia 114: 29–37.

GEITLER, L. 1977. Zur Entwicklungsgeschichte der Epithemiaceen *Epithemia*, *Rhopalodia*, und *Denticula* (Diatomophyceae) and ihre vermutlich symbiotischen Spharoidkorper. Plant Syst. Evol. 128: 259–275.

GREGORY, S. V. 1980. Effects of light, nutrients and grazing on periphyton communities in streams. Ph.D. dissertation, Oregon State University, Corvallis, OR. 151 p.

1983. Plant–herbivore interactions in stream systems, p. 159–189. *In* J. R. Barnes aand G. W. Minshall [ed.] Stream ecology: application and testing of general ecological theory. Plenum Press, New York, NY.

GRIMM, N. B., AND S. G. FISHER. 1986. Nitrogen limitation in a Sonoran desert stream. J. N. Am. Benthol. Soc. 5: 2–15.

HILL, W. R., AND A. W. KNIGHT. 1987. Experimental analysis of the grazing interaction between a mayfly and stream algae. Ecology 68: 1955–1965.

1988. Nutrient and light limitation of algae in two northern California streams. J. Phycol. 24: 125–132.

JACOBY, J. M. 1985. Grazing effects on periphyton by *Theodoxus fluviatilis* (Gastropoda) in a lowland stream. J. Freshwater Ecol. 3: 265–274.

JASPER, S., AND M. L. BOTHWELL. 1986. Photosynthetic characteristics of lotic periphyton. Can. J. Fish. Aquat. Sci. 43: 1960–1969.

LAMBERTI, G. A., L. R. ASHKENAS, S. V. GREGORY, A. D. STEINMAN, AND C. D. MCINTIRE. 1989. Productive capacity of periphyton as a determinant of plant–animal interactions in streams. Ecology 70: 1840–1856.

LAMBERTI, G. A., AND V. H. RESH. 1983. Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. Ecology 64: 1124–1135.

LIAO, C. F.-H. 1977. The effect of nutrient enrichment on nitrogen fixation activity in the Bay of Quinte, Lake Ontario. Hydrobiologia 56: 273–279.

LOWE, R. L., S. W. GOLLADAY, AND J. R. WEBSTER. 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. J. N. Am. Benthol. Soc. 5: 221–229.

LOWE, R. L., AND R. D. HUNTER. 1988. Effect of grazing by *Physa integra* on periphyton community structure. J. N. Am. Benthol. Soc. 7: 29–36.

LUBCHENCO, J., AND J. CUBIT. 1980. Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. Ecology 61: 676–687.

MAZUMDER, A., W. D. TAYLOR, D. J. MCQUEEN, AND D. R. S. LEAN. 1989. Effects of nutrients and grazers on periphyton phosphorus in lake enclosures. Freshwater Biol. 22: 405–415.

MCCORMICK, P. V., AND R. J. STEVENSON. 1989. Effects of snail grazing on benthic community structure in different nutrient environments. J. N. Am. Benthol. Soc. 8: 162–172.

MOORE, J. W. 1975. The role of algae in the diet of *Asellus aquaticus* L. and *Gammarus pulex* L. Ecology 44: 719–730.

MORRISON, D. F. 1967. Multivariate statistical methods. McGraw Hill, New York, NY. 338 p.

MULHOLLAND, P. J., J. D. NEWBOLD, J. W. ELWOOD, AND C. L. HOM. 1983. The effect of grazing intensity on phosphorus spiralling in autotrophic streams. Oecologia 53: 358–366.

MULHOLLAND, P. J., A. D. STEINMAN, A. V. PALUMBO, J. W. ELWOOD, AND D. B. KIRSCHTEL. 1991. Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. Ecology 72: 966–982.

ODUM, E. P., E. J. KUENZLER, AND M. X. BLUNT. 1958. Uptake of P^{32} and primary productivity in marine benthic algae. Limnol. Oceanogr. 3: 340–345.

PAUL, B. J., AND H. C. DUTHIE. 1989. Nutrient cycling in the epilithon of running waters. Can. J. Bot. 67: 2302–2309.

PETERSON, B. J., J. E. HOBBIE, T. L. CORLISS, AND K. KRIET. 1983. A continuous-flow periphyton bioassay: tests of nutrient limitation in a tundra stream. Limnol. Oceanogr. 28: 583–591.

PETERSON, B. J., J. E. HOBBIE, A. E. HERSHEY, M. A. LOCK, T. E. FORD, J. R. VESTAL, V. L. MCKINLEY, M. A. J. HULLEN, M. C. MILLER, R. M. VENTULLO, AND G. S. VOLK. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science (Wash., DC) 22: 1383–1386.

PORTER, K. G. 1973. Selective grazing and differential digestion of algae by zooplankton. Nature (Lond.) 244: 179–180.

POWER, M. E., W. J. MATTHEWS, AND A. J. STEWART. 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. Ecology 66: 1448–1456.

PRINGLE, C. M. 1987. Effects of water and substratum nutrient supplies on lotic periphyton growth: an integrated bioassay. Can. J. Fish. Aquat. Sci. 44: 619–629.

1990. Nutrient spatial heterogeneity: effects on community structure, physiognomy, and diversity of stream algae. Ecology 71: 905–920.

RIBER, H. H., AND R. G. WETZEL. 1987. Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton. Limnol. Oceanogr. 32: 1181–1194.

SCHINDLER, D. W. 1971. Carbon, nitrogen, phosphorus and the eutrophication of freshwater lakes. J. Phycol. 7: 321–329.

STEINMAN, A. D., C. D. MCINTIRE, S. V. GREGORY, AND G. A. LAMBERTI. 1989. Effects of irradiance and grazing on lotic algal assemblages. J. Phycol. 25: 478–485.

STEINMAN, A. D., C. D. MCINTIRE, S. V. GREGORY, G. A. LAMBERTI, AND L. R. ASHKENAS. 1987. Effects of herbivore type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. J. N. Am. Benthol. Soc. 6: 175–188.

STEINMAN, A. D., P. J. MULHOLLAND, A. V. PALUMBO, T. F. FLUM, J. W. ELWOOD, AND D. L. DEANGELIS. 1990. Resistance of lotic ecosystems to a light elimination disturbance: a laboratory stream study. Oikos 58: 80–90.

STENECK, R. S., AND L. WATLING. 1982. Feeding capabilities and limitations of herbivorous molluscs: a functional group approach. Mar. Biol. 68: 299–319.

STERNER, R. 1986. Herbivores' direct and indirect effects on algal populations. Science (Wash., DC) 231: 605–607.

STEVENSON, R. J. 1984. Procedures for mounting algae in a syrup medium. Trans. Am. Microsc. Soc. 103: 320–321.

STEWART, A. J. 1987. Responses of stream algae to grazing minnows and nutrients: a field test for interactions. Oecologia 72: 1–7.

STOCKNER, J. G. 1971. Ecological energetics and natural history of *Herdriodiscus truquii* (Diptera) in two thermal spring communities. J. Fish. Res. Board Can. 28: 73–94.

STOCKNER, J. G., AND K. R. S. SHORTREED. 1978. Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island. J. Fish. Res. Board Can. 35: 28–34.

1988. Response of *Anabaena* and *Synechococcus* to manipulation of nitrogen:phosphorus ratios in a lake fertilization experiment. Limnol. Oceanogr. 33: 1348–1361.

SUMNER, W. T., AND C. D. MCINTIRE. 1982. Grazer–periphyton interactions in laboratory streams. Arch. Hydrobiol. 93: 135–157.

U.S. ENVIRONMENTAL PROTECTION AGENCY (USEPA). 1983. Methods for chemical analysis of water and wastes. EPA-600/4-79-020, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

WINTERBOURN, M. J. 1990. Interactions among nutrients, algae, and invertebrates in a New Zealand mountain stream. Freshwater Biol. 23: 463–474.

Organochlorine Concentrations in the Plankton of Lakes in Southern Ontario and Their Relationship to Plankton Biomass

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Hydrophobic organochlorine contaminants were surveyed in net plankton from 33 lakes in southern Ontario. Organochlorines were detected in all lakes. The relative concentrations of 12 common organochlorines were generally similar, suggesting that they derive from a common source, probably atmospheric transport. Variability in relative abundances of these contaminants increased with contaminant concentration, while contaminant concentration was related to plankton biomass, total phosphorus, and conductivity. Lakes with low plankton biomass had high organochlorine concentrations in that biomass, indicating that a large portion of the variation among lakes in the concentration of contaminants in plankton can be ascribed to variable planktonic biomass. This negative relationship with plankton biomass, or biomass dilution effect, was strongest for the more hydrophobic and refractory compounds.

On a procédé au relevé des concentrations de contaminants organochlorés hydrophobes dans le plancton tamisé de 33 lacs du sud de l'Ontario. La présence des composés organochlorés a été détectée dans tous les lacs. En général, 12 composés organochlorés communs avaient une concentration relative semblable; cela pourrait indiquer qu'ils proviennent d'une source commune, probablement le transport atmosphérique. La variabilité de l'abondance relative de ces contaminants s'accroissait selon la concentration des contaminants alors que cette dernière était en rapport avec la biomasse planctonique, le phosphore total et la conductivité. Dans les lacs à faible biomasse planctonique, on observait une forte concentration de composés organochlorés dans cette biomasse; cela indique qu'une partie importante de la variation entre lacs de la concentration des contaminants dans le plancton est attribuable aux variations de la biomasse planctonique. Cette relation inverse avec la biomasse planctonique, ou effet de dilution dans la biomasse, était le plus accentuée dans le cas des composés qui étaient le plus hydrophobes ou réfractaires.

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Contamination of aquatic ecosystems by organochlorines has occurred on a global scale; these compounds can be readily detected in precipitation and surface waters throughout the Northern Hemisphere (e.g. Risebrough et al. 1968; Bidleman and Onley 1973; Bidleman et al. 1989; Harvey and Steinhauer 1974; Harding 1986; Day 1990).

In the Laurentian Great Lakes and in smaller lakes within the Great Lakes drainage basin, there is considerable variation among lakes in the concentration of contaminants in fish tissue and other biological materials (e.g. Stevens and Neilson 1989; Rasmussen et al. 1990), although the differences between different components of the fauna may not be consistent among lakes (Macdonald and Metcalfe 1991). In the Great Lakes, variations in concentration with time have also been reported (Bauman and Whittle 1988; Devault et al. 1988). Long-range transport and deposition via precipitation is thought to be the most important source of these organochlorines (Strachan and Huneault 1979; Johnson et al. 1988; Swackhammer et al. 1988; Arimoto 1989; Macdonald and Metcalfe 1991).

Yan et al. (1989) were able to relate differences in metal levels in the plankton of Ontario lakes to chemical and biological attributes of those lakes. Similarly, one might expect that, within a restricted geographic area with similar inputs from precipitation, interlake differences in concentrations of organochlorines should depend on morphometric, chemical, hydrological, and biological differences among the lakes (Macdonald and Metcalfe 1991). Rasmussen et al. (1990) have recently presented evidence in support of a strong role for food web structure in determining interlake differences in organochlorine levels in fish.

Highly hydrophobic contaminants in lake water are concentrated on and in plankton organisms, and their concentration is magnified by trophic interactions (e.g. Harding 1986; Bauman and Whittle 1988). The concentration of truly dissolved con-

taminants may be relatively small. If hydrophobic contaminants in lake water are mostly associated with plankton, and if the volumetric concentration of those contaminants is similar among lakes (as one might expect for lakes within a restricted area if the atmosphere is the common and dominant source), then the contaminant concentration per unit biomass might be negatively related to biomass concentration. That is, lakes with more plankton biomass might tend to have less contaminant per unit biomass. Conversely, when biomass is low, contaminant concentration per unit biomass should be high. This suggestion is related to the observation that particulate material in water can reduce bioaccumulation of hydrophobic contaminants by fish (e.g. Shrap and Opperhuizen 1990), in that it is also likely that as particulate matter increases, the concentration of truly dissolved contaminants goes down, as does the amount of contaminant per unit mass of particles. It is concentration per unit biomass that is usually reported for hydrophobic contaminants, not concentration per unit volume, and perhaps this practice tends to emphasize contaminant concentrations in oligotrophic systems.

The goals of this study were to examine this biomass dilution hypothesis by measuring the concentration of hydrophobic organochlorines in the net plankton of lakes which have no known direct sources of contaminants and to look for other correlates of organochlorine concentration. The lakes were chosen to minimize effects of morphometry; they are small to medium sized (1.8–18 km^2) and more than 20 m deep. They are from a restricted geographic area and were sampled within a 2-wk period during fall mixis. In some instances, our "lakes" are well-isolated basins of the same lake. Although our hypothesis might be relevant to other components of the biota besides net plankton, we chose to work with net plankton because it can be quantitatively sampled in a uniform manner to yield sufficient material for contaminant analysis.

Materials and Methods

The 33 lake basins sampled (Table 1) were part of an ongoing study of lake food webs and trophic status; they were visited for contaminant samples during fall overturn, November 13–27, 1986. Net hauls were made with a 0.5-m-diameter plankton net with a 150-μm mesh. The net was pulled from 2 m above the bottom to the surface where the depth was at least 17 m. The number of hauls was dependent on the amount of material obtained per haul, but the distance passed by the net was recorded so that the volume sampled could be calculated assuming 100% net efficiency.

Conductivity was measured with a Y.S.I. conductivity meter. Total phosphorus was measured on 0–10 m integrated samples collected during the previous or following August. Phosphorus was measured as phosphate using the ascorbic acid/molybdenum method following digestion of the samples with perchloric acid in an autoclave.

Samples were frozen on dry ice and remained frozen until they were processed. Samples were then freeze-dried and extracted by soxhlet extraction using dichloromethane. The soxhlet extractors were prepared by preextracting a small quantity of Celite 545 in a glass thimble with dichloromethane. The weighed sample was then added to the thimble and extracted with fresh dichloromethane for at least 20 cycles. After extraction, the extract volumes were reduced to 10–15 mL and stored cold until cleanup. To facilitate analysis, extracts were cleaned up by gel permeation chromatography using a mixture of hexane–dichloromethane (55:45, v:v) as eluting solvent. A chromotagraphic column (2 cm outside diameter) was plugged with teflon wool and filled to a length of 50 cm with preswelled Bio-Beads S-X3 (200–400 mesh; Bio-Rad Laboratories, Richmond CA). A 250-mL solvent reservoir was maintained full to give a flow rate of 1.5 $mL \cdot min^{-1}$ through the column. Under conditions of gravity flow, the first 110 mL contain nearly all of the lipids. This fraction was collected and evaporated under vacuum to a constant weight to give total lipid weight in the sample. The next 110 mL contain the organochlorines. This fraction was collected and evaporated to 10–15 mL under vacuum, and 2 mL of 2,2,4-trimethylpentane was added as a keeper. Residual lipids were removed by passing the extract through a minicolumn consisting of a Pasteur pipet plugged with teflon wool and packed with several grams of 60% (w:w) H_2SO_4 on silica gel on top of several grams of Fluorosil. The column was rinsed with 15 mL of 155 dichloromethane in hexane and the extract was reduced at room temperature to a final volume of 1 mL using a jet of nitrogen gas.

Extracts were analyzed by dual-column capillary gas chromatography on a Varian 4600 gas chromatograph. A DB-5 capillary column was used for quantification, while confirmations were obtained with a DB-17 column. After a 2-min initial hold at 90°C, the oven temperature was programmed from 90 to 270°C at $2°C \cdot min^{-1}$ where it was held for 25 min. Helium was the carrier gas and nitrogen was used as a make-up gas at the detectors.

We attempted to quantify 12 common contaminants in all lakes. Some contaminants were not quantified in certain lakes because they were very low in concentration (four cases) and/or there were interfering substances (three cases). To facilitate statistical analysis, these seven missing values out of 396 (12 contaminants × 33 lakes) were estimated using multiple regression models based on the concentration of other pesticides or of other PCBs. For the low values, the regression models confirmed that the concentrations should be near zero given the concentrations of other contaminants.

All statistical analyses used Systat (Wilkinson 1984).

Results

The concentrations of the contaminants varied among the lakes (Table 2), but all contaminants tended to be positively correlated with each other, with r values ranging from 0.17 to 0.96. The pattern of contamination among the lakes can be illustrated with a principal components analysis based on the correlations among contaminants (Fig. 1). All of the organochlorines are positively correlated with principal components axis 1 (PC1; Table 3), so axis 1 can be taken as a general index of organochlorine contamination. Because using this index is more convenient than analyzing the different contaminants individually, and because simply adding the concentrations hides differences among contaminants and weights them by average concentration, we elected to use this measure to relate to other lake characteristics. The second principal component (PC2; Table 3) is positively correlated with all pesticides and pesticide derivatives and negatively correlated with all PCBs, so can be used in a similar fashion as an index of the relative abundance of these groups. Most (61%) of the variance in the data set is associated with PC1, while only 14% is associated with PC2. The general pattern is one of increasing variability in organochlorine composition with increasing organochlorine concentration (Fig. 1).

TABLE 1. Lakes surveyed for selected organochlorines, their location, total phosphorus (TP) concentration ($\mu g \cdot L^{-1}$), and conductivity (μmhos).

Lake	County	°N	°W	TP	Conductivity
1. Boshkung	Haliburton	45°04′	78°44′	4.6	37
2. Brulé	Frontenac	45°04′	76°26′	4.2	99
3. Buck (north bay)	Frontenac	44°31′	76°28′	10.9	80
4. Buck (south bay)	Frontenac	44°32′	76°26′	11.0	70
5. Buckshot	Frontenac	45°00′	77°04′	7.5	60
6. Canoe	Frontenac	44°35′	76°33′	7.7	106
7. Chandos	Peterborough	44°49′	78°00′	8.5	104
8. Crow	Frontenac	44°43′	76°37′	7.6	114
9. Desert	Frontenac	44°33′	76°35′	7.0	149
10. Devil	Frontenac	44°35′	76°27′	8.4	139
11. Drag	Haliburton	45°05′	78°24′	5.4	49
12. Eels	Bancroft	44°54′	78°08′	6.4	50
13. Halls	Haliburton	44°07′	78°45′	3.2	21
14. Jacks	Peterborough	44°44′	78°03′	10.0	112
15. Kashagawigamog	Haliburton	44°58′	78°35′	6.5	60
16. Koshlong	Haliburton	44°59′	78°29′	5.6	21
17. Knowlton	Frontenac	44°27′	76°37′	7.4	210
18. Little Hawk	Haliburton	45°09′	78°43′	2.8	19
19. Maple	Haliburton	45°06′	78°40′	6.5	49
20. Mazinaw (north basin)	Frontenac	44°55′	77°12′	4.3	58
21. Mazinaw (south basin)	Frontenac	44°53′	77°11′	5.0	56
22. Mississagagon	Frontenac	44°52′	77°06′	7.6	160
23. Mississagua	Peterborough	44°42′	78°19′	4.8	39
24. Mountain	Haliburton	44°59′	78°43′	3.9	41
25. Paudash (north bay)	Haliburton	44°59′	78°04′	6.1	62
26. Redstone	Haliburton	44°11′	78°32′	5.4	24
27. Salmon	Peterborough	44°49′	78°27′	6.3	155
28. Sharbot (west basin)	Frontenac	44°46′	76°41′	9.4	160
29. Shabomeka	Frontenac	44°54′	77°08′	6.8	61
30. Silver	Frontenac	44°50′	76°36′	10.9	212
31. St. Nora	Haliburton	45°09′	78°50′		
32. Sydenham	Frontenac	44°25′	76°33′	16.3	110
33. Wollaston	Hastings	44°51′	77°51′	8.0	140

To illustrate the strong correlation among the contaminants summarized by PC1, which is evidence of a common source which we believe to be the atmosphere, we plotted the concentrations of contaminants in contrasting lake pairs (Fig. 2). The first lake pair, Jacks (lake 14) and Mountain (lake 24), are at opposite ends of PC1 (Fig. 1), with Jacks being at the left and Mountain at the right. Both are near the middle of the plot on the vertical axis, PC2. Accordingly, since PC2 reflects composition, the correlation between the lakes in the concentration of the different contaminants is relatively good even though the lakes represent extremes with respect to overall contaminant concentration. In contrast, Drag (lake 11) and Boshkung (lake 1) are both to the right on PC1, with high organochlorine concentrations, but are high and low with respect to PC2. The correlation between their contaminants is still evident, but weaker. Lastly, the lowest panel of Fig. 2 contrasts Desert (lake 9) and Maple (lake 19), which are both within the cluster of lakes at the left-centre of the principal components plot, but very different with respect to location and conductivity. Their contaminants are strongly correlated except for DDD. In all of the comparisons illustrated in Fig. 2, we avoided using lakes where contaminants levels had been estimated by regression.

The level of organochlorine contamination in the lakes, as summarized by PC1, was negatively correlated with net plankton biomass (Fig. 3). The dilution hypothesis predicts that concentration should be proportional to the reciprocal of biomass. The relationship we observed was of this form, although we chose to illustrate the trend with a logarithmic rather than a reciprocal relationship because the reciprocal fit displayed a strong trend of increased magnitude of residuals as a function of 1/net biomass. Total phosphorus and conductivity were also correlated with PC1, but less strongly ($r = -0.36$ and $r = -0.37$).

The residual variation from the relationship between net plankton biomass and PC1 was related to PC2 ($r^2 = 0.41$), suggesting that the relationship of organochlorine concentrations to net plankton biomass was dependent on the relative amount of pesticides and PCBs in the organochlorine mixture. Accordingly, we repeated the analysis using the PCBs and the pesticides separately. Again, for both groups of organochlorines, PC1 was positively correlated with all of the original variables (Table 3). The relationship between PC1 and net plankton biomass was stronger for PCBs (Fig. 4) than for total organochlorines. Even including the two lakes (26, Redstone and 32, Sydenham) not used in the regression shown in Fig. 4, the coefficient of determination was 0.53. In contrast, the parallel analysis using only the pesticides was weaker ($r^2 = 0.28$; not illustrated).

Individual organochlorines were also correlated with net plankton biomass, with correlations (with the logarithm of biomass) ranging from 0.26 and 0.27 (lindane and BHC) to 0.82 (PCB153).

Discussion

The net plankton from all of the lakes had measurable concentrations of organochlorines. The concentrations observed were not dissimilar to those described for the oceanic waters

TABLE 2. Concentrations of contaminants in net plankton from the lakes. All are ng·g dry weight^{-1}. Estimated concentrations (see Materials and Methods) are indicated with asterisks. Lakes are numbered as in Table 1. Abbreviations for the contaminants are as follows: BHC, *a*-BHC (hexachlorobenzene); lind, lindane; a-ch, *a*-chlordane; g-ch, *g*-chlordane. DDE and DDD are two breakdown products of the pesticide DDT. The five numbered contaminants are polychlorinated biphenyls.

Lake	BHC	lind	a-ch	g-ch	DDT	DDD	DDE	101	118	138	153	180
1	1.1	0.5	13.5	5.5	25.3	14.2	42.3	3.0	3.4	3.7	6.3	1.6
2	2.6	4.4	7.3	3.1	22.7	20.9	44.3	0.9	1.9	2.3	3.2	0.8
3	1.0	1.1	5.6	2.4	6.2	5.0	24.8	0.7	1.0	0.8	0.8	0.3
4	0.9	1.1	4.5	1.1	6.3	8.9	9.1	0.7	1.4	1.3	1.9	0.7
5	0.7	1.6	11.5	2.4	10.0	20.1	11.1	1.5	0.3	0.6	0.7	0.5
6	0.7	1.2	13.1	6.4	40.4	6.6	42.8	1.2	1.7	3.9	4.1	0.8
7	6.2	3.8	3.8	2.1	10.7	19.9	11.9	0.7	0.3	1.1	1.6	0.5
8	2.3	3.2	7.9	2.6	15.8	19.9	11.9	0.7	0.3	1.3	2.1	0.5
9	1.1	2.9	12.6	5.2	21.9	7.0	67.5	1.0	1.1	1.6	2.1	0.8
10	1.9	3.6	8.3	3.3	12.6	22.9	37.4	0.8	0.7	1.1	1.5	0.4
11	10.3	7.6	24.9	12.1	40.9	52.7	75.4	1.3	1.9	2.7	3.4	0.7
12	4.2	3.7	4.9	0.9	7.6	6.9	9.4	0.6	0.8*	1.3	1.8	0.7
13	6.2	5.7	4.8	1.8	11.9	19.8	7.9	0.5	0.9	1.2	1.7	0.4
14	2.8	2.0	4.8	1.7	6.6	6.3	12.3	0.5	0.7	0.8	1.1	0.6
15	1.3	14.5	15.0	7.4	35.9	35.1	62.8	1.2	1.4	2.1	3.3	0.8
16	1.7	3.5	0.3	1.1	41.2	0.7	32.2	1.1	0.7	1.6*	2.3	1.3
17	1.5	2.8	11.1	4.3	15.3	9.0	60.8	0.9	0.9	1.0	1.4	0.6
18	3.8	3.9	9.4	2.8	20.4	21.3	9.5	1.3	2.1*	2.5	4.0	1.2
19	1.5	2.0	4.6	1.3	10.0	23.4	10.7	0.6	0.5	1.1	1.3	0.4
20	0.8	0.9	6.1	2.4	19.4	12.1	27.5	1.3	3.1	2.8	4.8	1.3
21	0.3	0.6	2.8	1.3	8.6	6.0	12.5	0.4	0.8	0.9	1.3	0.4
22	2.6	3.4	6.9	1.2	10.0	23.5	12.0	0.8	1.4	1.1	1.6	0.3
23	1.6	2.6	13.1	4.7	37.0	36.2	34.4	3.0	4.3	2.8	3.8	1.0
24	9.9	5.7	17.7	8.3	41.7	47.1	63.3	3.9	2.6	3.7	4.8	2.5
25	12.5	12.8	21.7	9.9	53.6	46.9	96.2	2.7	5.6	3.9	4.7	1.2
26	5.5	5.7	17.5	9.1	46.9	24.2	63.1	3.2	4.4	4.2	5.8	3.4
27	0.4	0.7	0.7	0.0*	7.8*	13.8	3.4	0.2	0.0*	0.2	0.3*	0.1
28	1.2	2.9	6.4	3.0	11.7	9.1	37.6	0.8	0.8	1.1	1.5	0.5
29	3.1	5.5	13.7	6.5	19.7	31.4	48.6	1.3	0.8	1.5	2.4	0.5
30	2.5	3.8	11.8	4.5	13.3	36.3	26.1	2.0	1.4	1.6	2.1	0.6
31	4.9	8.6	14.7	7.9	84.0	183.7	28.0	1.7	2.2	3.0	4.1	1.2
32	4.4	4.4	4.6	0.9	5.0	29.3	13.7	3.0	2.0	1.4	1.1	0.1
33	0.8	1.6	11.3	5.0	22.6	16.2	32.7	2.1	2.1	2.6	3.4	1.2
Mean	3.1	3.9	9.6	4.0	22.5	25.3	32.8	1.4	5.6	1.9	2.6	0.8

(Bidleman and Onley 1973; Harvey and Steinhauer 1974; Harding 1986) and to those previously measured for lakes in this area not contaminated except by the atmosphere (Macdonald and Metcalfe 1991). It is interesting that Macdonald and Metclafe (1991) found high levels of PCBs in smallmouth bass (*Micropterus dolomieui*) from Boshkung and St. Nora lakes, although not in net plankton. We did find that these lakes had relatively high levels of PCBs and other contaminants (Fig. 1, lakes 1 and 31). However, it must be emphasized that our estimates are single samples from lakes, and we do not claim to have characterized those lakes; we have no estimates of spatial or temporal variation.

As expected for contaminants delivered via long-range atmospheric transport, their concentrations were correlated. That the relative abundances of the contaminants became more variable as overall concentrations increased could indicate that some lakes had additional sources for some compounds. A second but compatible hypothesis is that lakes with high organochlorine concentrations are ones in which organochlorines are lost at slower rates, and therefore, variation in the relative importance of different loss processes (e.g. sedimentation, photolysis, anaerobic dechlorination, volatilization), that have different rates for different compounds, generates the differences in relative abundance (e.g. Larsson et al. 1990). In view of the fact that DDT breaks down to DDE and DDD, and that the rate of this transformation might vary among lakes, more variability in this family might be expected.

In agreement with our initial hypothesis, concentrations of organochlorines, expressed as contaminant per unit dry mass of plankton, were negatively correlated with plankton biomass measured as dry mass per unit volume. This inverse relationship of plankton biomass and degree of contamination by organochlorines was generally weaker for pesticides than for PCBs. We believe that this trend reflects differences in hydrophobicity and stability, but could also reflect greater variation in input, as discussed above. While the strength of the association between neutral organic contaminants and particulate matter in aquatic systems is known to be dependent on hydrophobicity of the compound and certain characteristics of the particle, such as carbon content, the importance of this association on a mass basis is directly dependent on the mass of the particulate phase (Allan 1986). Thus, for less hydrophobic contaminants such as *a*-BHC and lindane that are less strongly associated with particulate phases, we would expect their negative correlation with particulate biomass to be weaker.

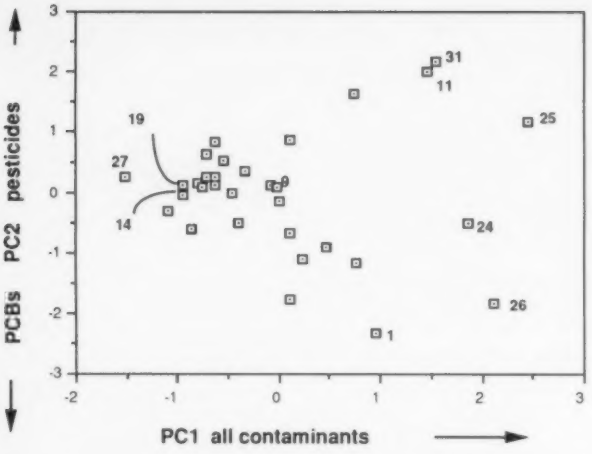

FIG. 1. Principal components ordination of the lakes based on the organochlorine concentrations in their plankton. Numbers identify lakes as in Table 1. Principal component loadings are provided in Table 3.

TABLE 3. Component loadings (correlations between the contaminants and principal components) and total variance explained by the components used to summarize contaminants. PC1 and PC2 use all 12 contaminants whereas PC1(pest.) and PC1(PCB) use only the first seven and last five contaminants, respectively.

Compound	PC1	PC2	PC1(pest.)	PC1(PCB)
a-BHC	0.629	0.393	0.712	
lindane	0.616	0.622	0.787	
a-Chlordane	0.873	0.189	0.902	
g-Chlordane	0.905	0.221	0.945	
DDT	0.843	0.178	0.839	
DDD	0.515	0.501	0.632	
DDE	0.778	0.136	0.810	
PCB101	0.758	−0.347		0.850
PCB118	0.822	−0.332		0.904
PCB138	0.905	−0.327		0.957
PCB153	0.862	−0.385		0.947
PCB180	0.747	−0.462		0.869
% variance	60.9	15.6	65.6	82.2

A complementary perspective on the inverse relationship between contaminant per unit biomass and biomass can be obtained from a theoretical framework concerning particle-size distribution and production by Borgmann (1982). This framework provides a means of relating the production at different trophic levels to a common basis so that, for example, one can compare the biomass of piscivores and planktivores in common units (the size-corrected biomass, F). The volume of pelagic zone required to produce an organism of a given size depends on trophic status and food web efficiency. Borgmann and Whittle (1983) used contaminant concentrations in fish to determine ecological efficiencies (or rather, particle-size conversion efficiencies) in Lake Ontario. They have exploited the phenomenon we have suggested here, that the levels of hydrophobic contaminants in biota are not only a measure of the concentration of contaminant in the water, but also of the volume of water that the biota in question represents.

Residual variation in the relationship between the most hydrophobic and stable of these compounds (i.e. the PCBs) and

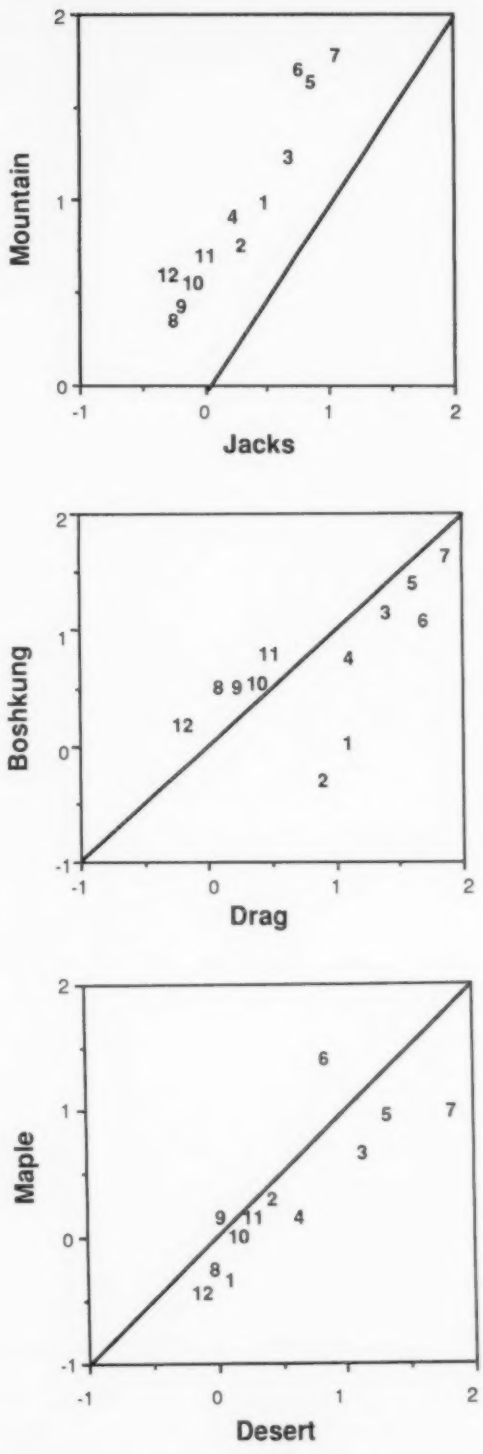

FIG. 2. Concentrations of contaminants (transformed to their logarithms) compared in three pairs of lakes from Table 1. Numbers refer to the contaminants as in Table 3. Lines indicate equal concentrations.

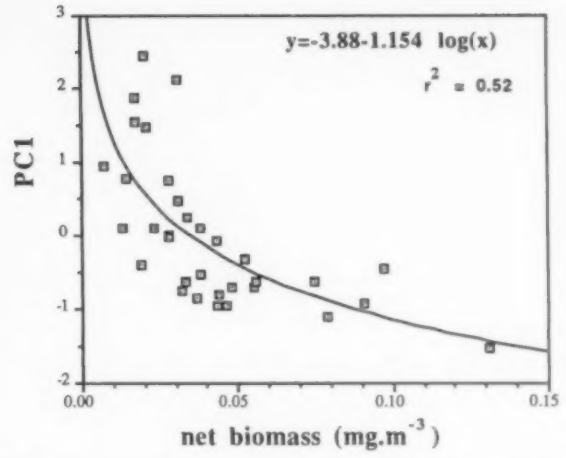

FIG. 3. Relationship between PC1 as illustrated in Fig. 1 and plankton biomass (dry weight).

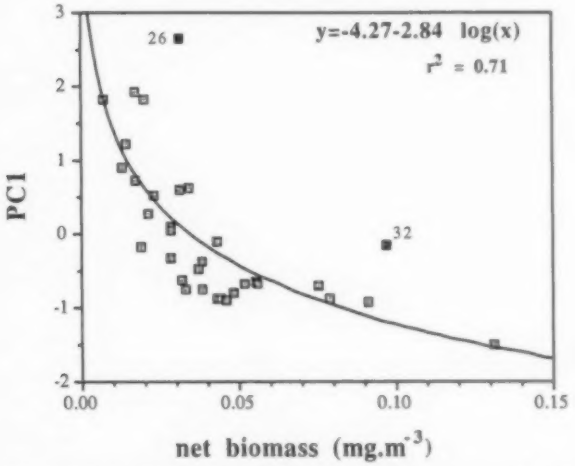

FIG. 4. Relationship between PC1 from a principal components analysis, using the PCBs only, and plankton biomass. Two lakes indicated, 26 (Redstone) and 32 (Salmon), were not included in the regression. Principal component loadings are provided in Table 3 (PC1(PCB)).

net plankton biomass may largely reflect error in the estimation of contaminant concentration and net biomass. For example, with a finer net (67 μm), we found net efficiency to vary between 20 and 50% for these lakes. The efficiency of the coarser net used here should be higher and less variable. If the residual is error, then we have underestimated the strength of the relationship between net biomass and contaminant concentration. Of course, there may be other factors which account for some of the residual variation, or which are important but correlated with net plankton biomass, inflating the apparent importance of that variable. Factors that might be correlated with net plankton biomass, but which are known to reduce bioaccumulation of contaminants by fish, include dissolved organic matter (e.g. Servos et al. 1989) and suspended sediments (e.g. Shrap and Opperhuizen 1990), and these may be acting to reduce contaminant bioaccumulation by zooplankton in these lakes, although none are strongly coloured or shallow.

Our results suggest that lakes that are more susceptible to contamination by hydrophobic contaminants can be recognized by their plankton biomass. A current concern, and one which was part of our motivation for this study, is the possibility that if biomass dilution of hydrophobic contaminants occurs, then phosphorus control and fish stocking might exacerbate contaminant problems in the Great Lakes. Both might lead to a reduction in plankton biomass and result in higher contaminant concentrations in that biomass and, ultimately, in aquatic vertebrates. Among this lake set, total phosphorus was negatively related to organochlorine concentrations in the net plankton, and we would predict that phosphorus control would increase contaminant concentrations in plankton and other biota dependent on the pelagic food web.

Stocking piscivorous fish in the Great Lakes is thought to have effects similar to phosphorus control; piscivores reduce planktivores, which in turn promotes large herbivorous zooplankton and results in reduced plankton biomass and water clarity (Kitchell et al. 1988). However, the effect of fish on plankton is largely one of changing size distribution (towards a domination by large zooplankton and algae) as well as reduced biomass (Mazumder et al. 1988). Size distribution also affects other processes such as sedimentation and light penetration (Mazumder et al. 1989, 1990), and changes in size distribution also mean changes in ecological efficiency, with implications for the efficiency of contaminant bioaccumulation, as noted above. The least contaminated lake in our data set, Salmon Lake, is not extreme in its summer phosphorus level, but is exceptional for its strong spring to summer decline in phosphorus, high net plankton biomass, and abundant large *Daphnia* (unpublished data). Similarly, Rasmussen et al. (1990) found that lakes without forage fish had lower organochlorine concentrations in lake trout (*Salvelinus namaycush*). Therefore, we suggest that nutrient loading and food web determinants of trophic status may have different and interacting effects on contaminant bioconcentration. These results concerning the relationship of net plankton biomass to the concentration of organochlorines in that biomass are a contribution to understanding these effects.

Acknowledgments

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References

ALLAN, R. J. 1986. The role of particulate matter in the fate of contaminants in aquatic systems. Inland Waters Directorate Scientific Series No. 142, Environment Canada, Ottawa, Ont. 128 p.

ARIMOTO, L. 1989. Atmospheric deposition of chemical contaminants to the Great Lakes. J. Great Lakes Res. 15: 339–356.

BAUMAN, P. C., AND D. M. WHITTLE. 1988. The status of selected organics in the Laurentian Great Lakes: an overview of DDT, PCBs, dioxins, furans, and aromatic hydrocarbons. Aquat. Toxicol. 11: 241–257.

BIDLEMAN, T. F., AND C. E. ONLEY. 1973. Chlorinated hydrocarbons in the Sargasso sea atmosphere and surface waters. Science (Wash., DC) 183: 516–518.

BIDLEMAN, T. F., ET AL. 1989. Toxaphene and other related organochlorines in Arctic Ocean fauna: evidence for atmospheric delivery. Arctic 42: 307–313.

BORGMANN, U. 1982. Particle-size conversion efficiency and total animal production in pelagic ecosystems. Can. J. Fish. Aquat. Sci. 39: 668–674.

BORGMANN, U., AND D. M. WHITTLE. 1983. Particle-size conversion efficiency and contaminant concentrations in Lake Ontario biota. Can. J. Fish. Aquat. Sci. 40: 328–336.

DAY, K. E. 1990. Pesticide residues in freshwater and marine zooplankton: a review. Environ. Pollut. 67: 205–222.

DEVAULT, D. S., J. M. CLARK, G. LAHVIS, AND J. WEISHAAR. 1988. Contaminants and trends in fall run coho salmon. J. Great Lakes Res. 14: 23–33.

HARDING, G. C. 1986. Organochlorine dynamics between zooplankton and their environment, a reassessment. Mar. Ecol. Prog. Ser. 33: 167–191.

HARVEY, G. R., AND W. G. STEINHAUER. 1974. Atmospheric transport of polychlorinated biphenyls to the North Atlantic. Atmos. Environ. 8: 777–782.

JOHNSON, M. G., J. R. M. KELSO, AND S. E. GEORGE. 1988. Loadings of organochlorine contaminants and trace elements to two Ontario lake systems and their concentrations in fish. Can. J. Fish. Aquat. Sci. 44: 3–13.

KITCHELL, J. F., M. S. EVANS, D. SCAVIA, AND L. B. CROWDER. 1988. Regulation of water quality in Lake Michigan: report of the Food Web Workshop. J. Great Lakes Res. 14: 109–114.

LARSSON, P., L. OKLA, S.-O. RYDING, AND B. WESTÖÖ. 1990. Contaminated sediment as a source of PCBs in a river system. Can. J. Fish. Aquat. Sci. 47: 746–754.

MACDONALD, C. R., AND C. D. METCALFE. 1991. Concentration and distribution of PCB congeners in isolated Ontario lakes contaminated by atmospheric deposition. Can. J. Fish. Aquat. Sci. 48: 371–381.

MAZUMDER, A., D. J. MCQUEEN, W. D. TAYLOR, AND D. R. S. LEAN. 1988. Effects of fertilization and planktivorous fish (yellow perch) on size distribution of particulate phosphorus and assimilated phosphate: large enclosure experiments. Limnol. Oceanogr. 33: 421–430.

MAZUMDER, A., W. D. TAYLOR, D. J. MCQUEEN, AND D. R. S. LEAN. 1989. Effects of fertilization and planktivorous fish on epilimnetic phosphorus and phosphorus sedimentation in large enclosures. Can. J. Fish. Aquat. Sci. 46: 1735–1742.

1990. Effects of fish and plankton on lake temperature and mixing depth. Science (Wash, DC) 247: 312–315.

RASMUSSEN, J. B., D. J. ROWAN, D. R. S. LEAN, AND J. H. CAREY. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can. J. Fish. Aquat. Sci. 47: 2030–2038.

RISEBROUGH, R. W., R. J. HUGGETT, J. J. GRIFFIN, AND E. D. GOLDBERG. 1968. Pesticides: transatlantic movements in the northeast trades. Science (Wash, DC) 159: 1233–1235.

SERVOS, M. R., D. C. G. MUIR, AND G. R. B. WEBSTER. 1989. The effect of dissolved organic matter on the bioavailability of polychlorinated dibenzo-*p*-dioxins. Aquat. Toxicol. 14: 169–184.

SHRAP, S. M., AND A. OPPERHUIZEN. 1990. Relationship between bioavailability and hydrophobicity: reduction of the uptake of organic chemicals by fish due to the sorption on particles. Environ. Toxicol. Chem. 9: 715–724.

STEVENS, R. J. J., AND M. A. NEILSON. 1989. Inter- and intralake distributions of trace organic contaminants in surface waters of the Great Lakes. J. Great Lakes Res. 15: 377–393.

STRACHAN, W. M. J., AND H. HUNEAULT. 1979. Polychlorinated biphenyls and organochlorine pesticides in Great Lakes precipitation. J. Great Lakes Res. 5: 61–68.

SWACKHAMMER, D. L., B. D. MCVEETY, AND R. A. HITES. 1988. Deposition and evaporation of polychlorobiphenyl congeners to and from Siskwit Lake, Isle Royale, Lake Superior. Environ. Sci. Technol. 22: 664–671.

WILKINSON, L. 1984. SYSTAT: the system for statistics. Systat Inc., Evanston, IL.

YAN, N. D., G. L. MACKIE, AND D. BOOMER. 1989. Chemical and biological correlates of metal levels in crustacean zooplankton from Canadian Shield lakes: a multivariate analysis. Sci. Total Environ. 87/88: 419–438.

Patterns and Limitations in Limnoplankton Size Spectra

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Ahrens, M. A., and R. H. Peters. 1991. Patterns and limitations in limnoplankton size spectra. Can. J. Fish. Aquat. Sci. 48: 1967–1978.

The hypothesis that biomass is uniformly distributed over logarithmic size classes was evaluated with plankton samples from 15 temperate lake sites in southern Quebec. Over the size range from 0.2 to 1600 μm equivalent spherical diameter (ESD), biomass tended to increase between logarithmic size classes at a median rate of 7%, and numerical abundance declined strongly with increasing size. The slope of the normalized biomass spectrum (reflecting overall trends in the distribution) becomes significantly steeper as total phosphorus decreases and total biomass declines. Hence, more oligotrophic systems have a more uniform biomass distribution and proportionately more small organisms. Over the observed size range, most samples were dominated by a phytoplankton mode between 20 and 50 μm ESD. Because of this dominance and because of the variability of the samples, most sample distributions could not be shown to differ significantly from unimodal lognormal distributions. Efforts to fit bimodal and trimodal distributions met with limited success, likely because only 39 size classes could be used. Simple mathematical descriptors of size spectra are insensitive to substantial departures from both linear and lognormal models, and therefore, variaion in the parameters of such descriptions are ineffective correlates of departures from average patterns.

L'hypothèse voulant que la biomasse soit uniformément répartie entre différentes classes de taille logarithmiques a été évaluée par échantillonnage du plancton dans quinze lacs tempérés du sud du Québec. Cet échantillonnage a montré que, pour une gamme de tailles allant de 0,2 à 1 600 μm de diamètre sphérique équivalent (DSE), la biomasse tend à s'accroître entre les classes de taille logarithmiques à un taux médian de 7 % alors que l'abondance numérique décline fortement avec l'augmentation de la taille. La pente de la courbe des classes normalisées de la biomasse (qui reflète les tendances d'ensemble de la distribution) s'accentue notablement à mesure que le phosphore total et la biomasse totale diminuent. Les systèmes les plus oligotrophes présentent donc une dstribution plus uniforme de la biomasse et contiennent proportionnellement plus de petits organismes. Pour l'ensemble de la gamme de tailles observée,la plupart des échantillons contenaient surtout du phytoplancton entre 20 et 50 μm de DSE. Du fait de cette dominance et à cause de la variabilité des échantillons, il n'a pas été démontré que la plupart des distributions corstatées à l'échantillonnage différaient substantiellement des distributions lognormales unimodales. Les efforts déployés pour vérifier les distributions bimodales et trimodales n'ont guère donné de résultats, probablement parce qu'on n'a pu les fonder que sur 39 classes de taille. Les descripteurs mathématiques simples de la gamme des tailles ne sont pas suffisamment sensibles pour mettre en évidence les écarts notables avec les modèles linéaire et lognormal, ce qui fait que les variations dans les paramètres de telles descriptions ne permettent pas d'établir des corrélations valables pour les écarts avec les distributions moyennes.

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The hypothesis that aquatic biomass is roughly uniformly distributed over logarithmic size classes (Sheldon et al. 1972) has received support from studies in the sea (Rodriguez and Mullin 1986) and freshwater (Sprules et al. 1983; Peters 1983a, 1985; Mazumder et al. 1988). This work also showed local modes in both pelagic and benthic distributions (Schwinghamer 1981, 1983; Sprules and Munawar 1986; Warwick and Joint 1987; Hanson et al. 1989; Echevarria et al. 1990), apparently corresponding to major functional groups, such as phytoplankton or zooplankton. Theoretical models of the plankton community (Platt and Denman 1978; Borgmann 1982, 1987; Dickie et al. 1987a, 1987b) have been encouraged by these regularities in the size structure and by the well-established size dependencies of many biological processes (Banse and Mosher 1980; Peters 1983b).

The development of ecologically practical allometric models has been hampered by the paucity of data, the small number of size classes enumerated, and the simplicity of current models. For example, the "normalized spectrum" (Platt and Denman 1978; Sprules and Munawar 1986) characterizes size distributions with only two parameters, but substantial differences in the detailed size structure may be indistinguishable at this level of abstraction. Peters (1983a) and Mazumder et al. (1988) used cumulative particulate phosphorus concentration as an index of biomass in successive size classes to develop linear models of community size structure, but particulate phosphorus has not been shown to be a consistent measure of biomass in individual size classes; moreover, their approach requires rather broad size classes to provide enough material for chemical analysis, and so does not support very detailed models. Yet, detailed models may be required before size structure analysis becomes a useful tool to predict contaminant fates (Griesbach et al. 1982; Borg-

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mann 1987), fishery yields (Dickie et al. 1987a, 1987b), or the impact of eutrophication on size structure (Peters 1983a, 1985; Sprules and Munawar 1986).

This paper presents a test of the uniform distribution hypothesis with limnoplankton samples from 15 Quebec lakes and an examination of the number, relative sizes, and positions of local modes in those distributions. In particular, attention is paid to the variability of the parameters of the relations used to describe size distributions because low variability would indicate that the models are insensitive to differences among lakes. Such insensitivity would greatly reduce the possibility that variations in the parameters of size distribution might be used to predict variations in community physiology. To the extent that parameters vary, several predictive relationships were developed and then evaluated using an independent data set. The data were also used to assess the use of particulate phosphorus as an index of biomass in different size classes.

Methods

Sampling

Fifteen lake sites, representing a broad range of phosphorus and chlorophyll concentrations (Table 1), were visited over the growing seasons of 1986 and 1987. Integrated epilimnetic water samples (at least 20 L per site) were collected during the day through a 2.5-cm-diameter tube connected to a piston pump, a system which Pace (1986) found to collect phytoplankton and zooplankton effectively. Water samples were poured into 1- or 4-L bottles and kept in the shade during transit to a laboratory refrigerator (maximum duration 1 h). Samples for microscopic examination were preserved with Lugol's iodine for phytoplankton (size range 1–100 μm) or 5% formalin for zooplankton (80–1500 μm). In 1987, subsamples preserved in formalin were also used to enumerate bacteria (0.2–1.5 μm).

Size Composition

The size distribution of plankton throughout the range 0.2–1500 μm equivalent spherical diameter (ESD) was determined by microscopy. The abundance and approximate diameters of bacteria were determined by epifluorescence microscopy with DAPI stain (Porter and Feig 1980) of samples preserved in formalin. Larger picoplankton were measured at 1250× on an inverted microscope, using samples preserved in Lugol's. Nannoplankton were measured at 1000× and 400× (Lund et al. 1958). Zooplankton were measured at 100× on the inverted microscope and at 40× under a dissecting microscope, using both samples preserved in Lugol's and those preserved in formalin. Individual volumes of organisms larger than 5 μm greatest axial linear dimension (GALD) were estimated by measuring length and width and taking the volume of similar regular geometric shapes as approximations. Smaller organisms were considered as spheres and classed by diameter into nine size intervals between 0.2 and 5 μm. The five magnifications used in this study allowed sufficient overlap between successive selection curves that, for many sizes, a choice was required between the counts at the two magnifications. In every case, the highest count was selected, on the assumption that lower counts were the result of partial selectivity.

Chemical Analyses

Epilimnetic concentrations of chlorophyll, total phosphorus, and seston dry weight were measured as potential predictors of the parameters of the size distributions. Total phosphorus concentrations and particulate phosphorus in several size fractions, separated by stainless steel screens (130-, 80-, 40-μm porosity) and Nuclepore membrane filters (5- and 0.4-μm porosity), were measured in triplicate using the ascorbic acid modification of the molybdenum blue technique (Strickland and Parsons 1968) after digestion with potassium persulfate under pressure (Menzel and Corwin 1965). Whole-sample chlorophyll *a* concentrations were also measured in triplicate (Strickland and Parsons 1968). Epilimnetic dry weights were determined by pouring a known volume of lake water through predried (60°C), weighed GFC filters and reweighing the filters after they had dried for 24 h to determine the weight of seston by difference.

TABLE 1. Southern Quebec lakes sampled in 1987, in order of increasing total phosphorus. Concentrations are for total epilimnetic samples ($mg \cdot m^{-3}$, with SD in parentheses). Dates are day of the year. Central, North, South, and Newport are basins of Lake Memphremagog.

Lake	Latitude	Longitude	No. of samples	Range of dates	Total phosphorus	Total chlorophyll
Bowker	45°25′	72°15′	1	183	3.50	1.39
Stukely	45°20′	72°15′	2	193–230	5.53 (1.53)	1.23 (1.40)
Orford	45°15′	72°20′	5	128–237	7.61 (2.90)	1.19 (0.50)
Lyster	45°04′	71°55′	3	149–227	10.16 (5.01)	1.44 (0.91)
Baldwin	45°04′	71°54′	3	149–227	14.23 (1.57)	2.10 (0.73)
O'Malley	45°13′	72°20′	1	177	14.41	4.01
Central	45°10′	72°15′	8	114–228	14.46 (3.03)	1.97 (0.89)
Cerises	45°18′	72°15′	2	193–236	14.74 (2.59)	3.82 (3.56)
North	45°15′	72°15′	5	120–226	15.03 (2.08)	2.18 (1.32)
Massawippi	45°15′	72°05′	1	177	15.52	2.55
Lovering	45°10′	72°10′	2	128–177	16.14 (3.01)	1.42 (0.44)
Argent	45°15′	72°25′	2	142–183	16.57 (0.71)	3.02 (2.01)
Trousers	45°10′	72°25′	1	181	17.77	3.38
South	45°00′	72°15′	6	114–235	19.27 (2.19)	3.87 (0.74)
Newport	44°55′	72°15′	4	128–204	20.70 (3.42)	3.16 (0.70)
Brome	45°15′	72°30′	1	142	20.82	3.15
Pond	45°10′	72°14′	3	183–224	24.59 (2.16)	2.54 (1.80)
Magog	45°20′	72°05′	4	128–223	32.61 (6.87)	5.01 (2.98)
Waterloo	45°20′	72°30′	4	142–229	81.72 (35.1)	17.64 (11.0)

Data Analyses

Computer programs were written to compute individual organism volumes, total counts and total volumes over sucessive size classes from length, width, and shape, microscopical magnifications, and water volumes. We elected to use size classes whereby the individual organism's volumes doubled in successive classes because wider classes would reduce the total number of classes available for each distribution and because narrower size classes would increase noise due to error in sizing and sampling. The geometric mean volume of organisms in the smallest interval was 0.0055 μm^3, corresponding to equivalent spherical diameters from 0.2 to 0.25 μm. The mean for the next interval was 0.0110 μm^3, corresponding to 0.25–0.31 μm ESD. In the largest interval counted (the 39th), the mean volume of 1.5×10^9 μm^3 includes organisms of between 1250 and 1600 μm ESD.

Curve Fitting

Statistical models of these size distributions can only be effective when there are samples with counts in most of the 39 size intervals. This was not always the case. Some samples could not be fully counted because abiotic particles obscured the field. In other samples, large peaks representing a bloom of a single taxon in one interval were followed by empty size classes. Finally, some oligotrophic lakes yielded too many zero counts in the zooplankton range. Although such problems could be addressed (e.g. rare organisms would be better represented by more intensive sampling), many cannot be dealt with after the fact. For the purpose of curve fitting, a total of 25 samples with "good coverage" were identified in the 1987 data set, where good coverage is defined as nonzero counts in at least 90% of the size classes, including at least some size classes above 600 μm ESD. Only samples meeting these criteria were used to estimate the parameters of size distribution models.

Such a selection biases the data set. For example, only 3 of the 29 samples collected in May and June, but 22 of the 30 samples collected in July and August, had "good coverage." Such biases may cause the selected data to be less representative of the size distributions in nature, although this cannot be ascertained because many of the samples with "poor coverage" could not be properly evaluated. Without this selection, the size distributions are so variable that they fit all models equally poorly and thus obscure differences in the models' effectiveness and potential power. This creates an asymmetry in assessing the fit of the models to the selected data. If the comparisons of different models were to identify one model as particularly effective, we could only consider that model as potentially strong, and must reserve judgement until more representative, unselected data are available to test the model further. However, if the assessment shows that the models cannot even discriminate given data with good coverage, then they certainly will not be able to do so when the coverage is poor and we can conclude that none of the models is likely to prove effective in future.

The simplest model evaluated was the normalized spectrum (Fig. 1), computed for each sample from the spectrum of volume concentrations in base 2 logarithmic size intervals. Such spectra plot the logarithm of the standardized abundances per interval, calculated as the volume concentration in the interval divided by the change in modal volume between intervals, on the ordinate versus the logarithm of size on the abscissa. The parameters of the straight line fitted to these points may be used

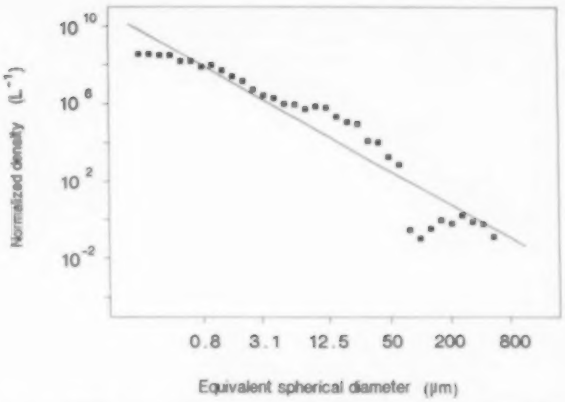

FIG. 1. Example of a normalized size-density spectrum (Lake Orford, July 22, 1987). The straight line is the least squares regression fitted to these data.

to compare samples. The intercept provides an estimate of relative abundance when mass (or volume) = 1 unit and the slope reflects the overall trend in mass or volume change from interval to interval. A significant slope indicates an overall trend with size, despite local peaks or troughs in the size spectrum. A slope of −1 indicates that biomass is approximately evenly distributed over size classes; steeper slopes (with larger absolute values) show that biomass declines with increasing size whereas shallower slopes reflect the reverse. Integration over any range of sizes provides a smoothed estimate of biovolume or biomass over that range.

Our data, like other normalized spectra (Sprules and Munawar 1986; Echevarria et al. 1990), show patterns in the residuals around the fitted curve, and hence the diagnostic statistics associated with the regression (r^2, F, SEE) will probably not be accurate. Nevertheless, if the spectra to be compared are computed over similar size ranges and have similar residual patterns, then the parameters may provide a basis for comparison of general trends in biomass distribution. If the normalized spectrum is an accurate representation of the data, then numerical integration under the fitted curve (multiplying together back-transformed ordinates and abscissas and summing) should provide an estimate of biomass similar to that observed over the same range. To test this hypothesis, the predicted integrated biovolumes from normalized spectra were regressed against observed total biovolumes. The results were also compared with those of Sprules and Munawar (1986) for Ontario lakes.

The finer structure of the distributions was examined in three stages involving models of increasing complexity. We first examined the possibility that plankton volume might be lognormally distributed over size. Since our size distributions are grouped by logarithmic (base 2) volume intervals, they should look like normal distributions when graphed (volume on the ordinate versus log size). A Kolmogoroff–Smirnoff test was used to test an overall lognormal (unimodal) fit to these data. We then tested the hypothesis that the distributions were bimodal, corresponding to two lognormal curves. If this hypothesis were true, then each distribution would be characterized by five independent parameters: two means, two variances, and a parameter between 0 and 1 describing the proportion of the total distribution in one of the modes. These parameters of the mixture density function were sought by a maximum likelihood

method (Clarke 1984; Ahrens 1989). Finally, a linearization technique for estimating the parameters of a nonlinear system (Draper and Smith 1981; Ahrens 1989) was used to estimate the eight independent parameters of trimodal fits.

Variability of Parameters

The heterogeneity among estimated parameters was evaluated in several ways. All pairwise comparisons were compared with the Least Significant Difference (LSD; 95% criterion) using Tukey's test based on the Studentized range, rather than the t distribution which would have increased the chance of falsely detecting "significant" differences (Type I error; John 1971). When linear regressions were compared, the models were first tested for homogeneity of variance by Bartlett's test, and if they passed this test, they were compared using an F test for the coincidence of two or more straight line regressions (Seber 1977).

Predictions

Predictive linear regression models, based on the data from 1987, were used to determine the correlation between various chemical and morphometric variables and the parameters of the size distributions. Regression was also used, where possible, to evaluate the correspondence between prediction and observation, using the independent daa set from 1986. The suitability of particulate phosphorus concentration as an index of size-specific biovolume was evaluated by comparing the ratio of those variables among size classes with analysis of variance (Seber 1977).

Results and Discussion

Detailed Size Structure

This analysis generated 20 size distributions in 1986 and 59 in 1987 spread over 15 lake sites, each distribution consisting of up to 39 size classes. Such data are too numerous to be detailed here, but are available in Ahrens (1989) and have been placed in the CISTI data bank.[2] In addition, they are available in both printed and electronic forms from the junior author (R.H.P.).

To visualize the general pattern of these distributions, the 1987 data were compiled to produce the "median distributions" of density and biomass, composed of the medians of all observed values for each size interval and approximate 95% limits around this median (Fig. 2). The distribution of numerical abundances across logarithmic size classes showed a fairly uniform decline as size increased (Fig. 2A). The median of the distribution of biomass (Fig. 2B and 2C) increased rapidly to about 1 μm ESD, then more gradually to about 30 μm, and was erratic beyond that point. On average, 50% of the community biomass was less than 45 μm ESD and 90% less than 700 μm (Fig. 2D). The summed biomass of this median distribution is 14.1 $mg \cdot L^{-1}$.

The median concentration of total phosphorus in our 15 lakes was 17.4 $\mu g \cdot L^{-1}$, so to compare our results with those of Sprules et al. (1983), representing less rich lakes (Table 2), the median biomass was determined for the 12 samples with lowest total phosphorus concentrations (median total phosphorus = 6.6 $\mu g \cdot L^{-1}$). Median summed biomass for these lakes was 6.2 $mg \cdot L^{-1}$. The peak in the distribution for these oligotrophic lakes occurred at 30 μm (Fig. 2E), half of the biomass was in particles smaller than this, and 90% of the bomass was in particles less than 175 μm ESD. Hence, these phosphorus-poor lakes had lower total plankton biomass concentrations and were dominated by smaller organisms than lakes with higher epilimnetic phosphorus concentrations. This pattern is consistent with trends in phytoplankton size (Watson and McCauley 1988; Sprules and Knoechel 1984).

Two lakes were sampled often enough throughout the season to examine temporal trends in the relative size. Table 3 provides some indication that biomass increased over the season, and in the more eutrophic Lake Magog, an increasing proportion of the total biomass lay in the zooplankton size range. Using all samples with good coverage of the size spectrum from all lakes, regression of the proportion of total biomass below 80 μm on day of the year shows a significant ($P < 0.001$), decreasing trend (slope = −0.0030), explaining 33% of the variation. Such a trend to increased size over the growing season would be expected on the basis of taxonomic analyses of successional trends in lakes (Bailey-Watts 1986; Sommer et al. 1986).

Trophic response models provide a crude basis of comparison for our total biomass results. Based on equations predicting the biomass of various components of the plankton from total phosphorus levels (summarized by Peters 1986), the summed biomasses predicted at 7 and 17 $\mu g\ P \cdot L^{-1}$ are 1.9 and 4.4 $mg \cdot L^{-1}$, respectively. These predictions are lower than our observed median values of 6.2 and 14.0 $mg \cdot L^{-1}$. Because the error associated with phosphorus response models is high, the median values we observed would lie within the summed 95% confidence limits of the predicted sums. Nevertheless, the differences are disturbingly large.

Our bacterial counts are similar to those obtained by other authors using similar methods (Hobbie et al. 1977); hence, we feel that our biomasses of bacteria and picoplankton are not likely to be methodological artifacts. Moreover, our estimates of zooplankton biomass are consistent with those predicted from the equations summarized in Peters (1986). If our data set is limited to low-phosphorus lakes and to the size range used by Sprules et al. (1983), i.e., $1 < ESD < 1500$ μm, the average integrated biomass (1.5 $mg \cdot L^{-1}$) reported by Sprules et al. (1983) is substantially lower than we measured (6.2 $mg \cdot L^{-1}$). Zooplankton biomasses are similar in both studies, but in their study, the total volume was approximately equally divided between the phytoplankton and zooplankton modes whereas the larger mode (i.e., zooplankton) usually represents less than 10% of the biomass in our samples. Their phytoplankton peaked on average at about 8 μm whereas the peak of the median distribution for our low-phosphorus lakes lies at about 30 μm (Fig. 2E). Thus, our size distributions indicate higher concentrations of larger phytoplankton than do previous studies.

Differences in phosphorus concentration are too small to produce this difference, and the larger and deeper lakes in our study (Table 2) should, if anything, have had depressed biomasses relative to expectation (Straskraba 1980). Therefore, neither nutrient levels nor lake morphology effectively explain these results. We feel that this difference is not artifactual because estimates of size-specific respiration of the phytoplankton (Ahrens 1989; Ahrens and Peters 1991) are consistent with expectation based on general allometric relations for unicellular organisms (Robinson et al. 1983). In addition,

[2]A complete set of tables is available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ont. K1A 0S2, Canada.

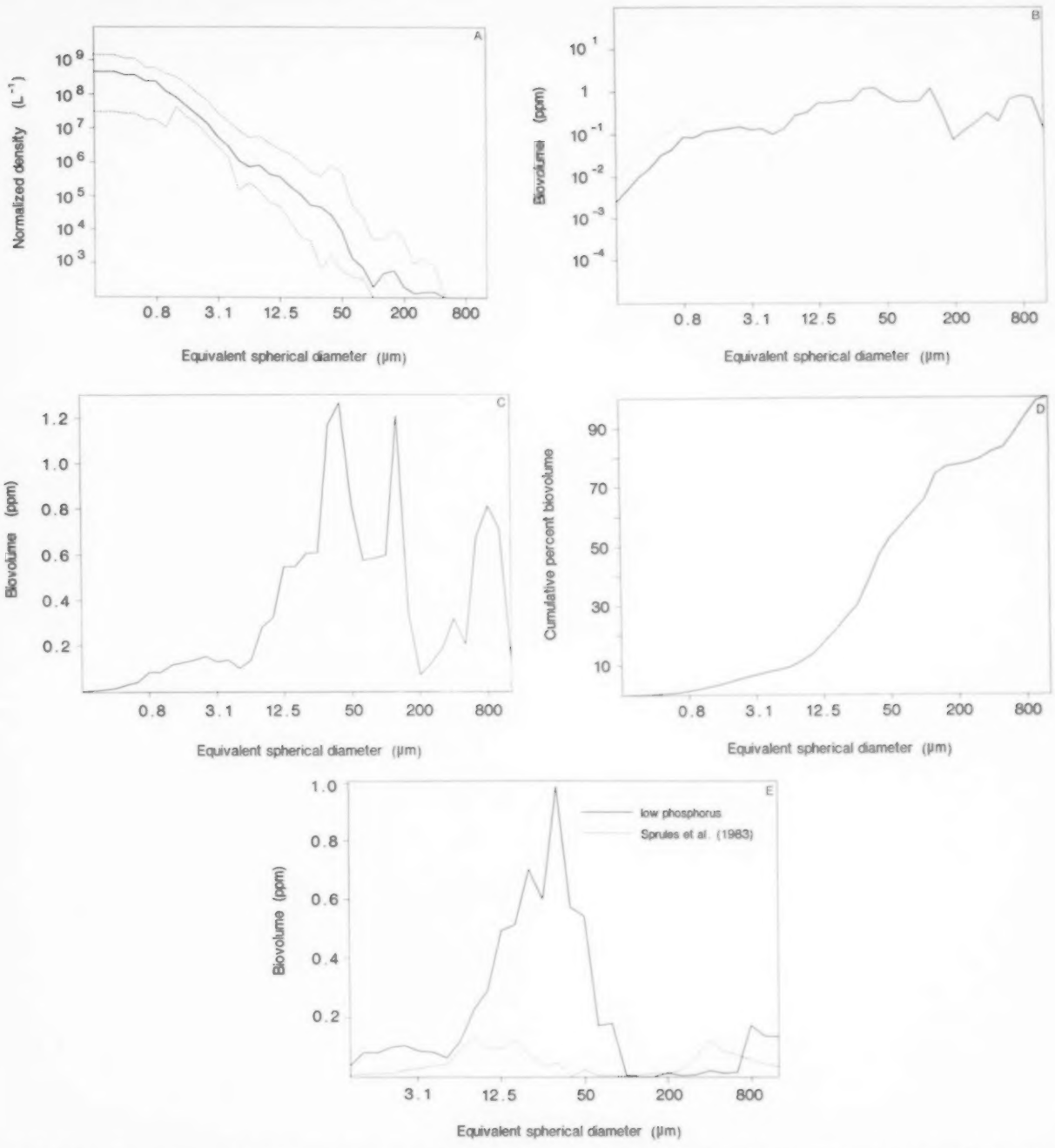

FIG. 2. Median distributions (n = 58) over logarithmic size classes of (A) organism density, (B) total organism volume on a logarithmic scale, (C) total organism volume on a linear scale, (D) cumulative total organism volume, and (E) low-phosphorus total organism volume. The 95% confidence intervals are shown on the first two panels. Parts per million (ppm) = $\mu^3 \cdot L^{-1} \times 10^{-9}$. The mean distribution obtained by Sprules et al. (1983) for low-phosphorus Ontario lakes is included in Fig. 2E.

an artifactual overestimate of microscopical biovolume should not be reflected in the chemical composition of physically fractionated samples of similar size, yet the ratio of particulate phosphorus to estimated biovolume for plankton between 5 and 80 μm is consistent with the ratios of other classes (see Table 9).

Although the difference between our results and those of Sprules et al. (1983) does not seem to be artifactual, it may be

TABLE 2. Comparisons between the physicochemical properties of the lakes we studied and those in Ontario studied by Sprules et al. (1983). The range and median are shown.

	This study		Sprules et al. (1983)
	All	Oliotrophic	
Total P ($mg \cdot m^{-3}$)	3–130 (17)	3–12 (7)	0.5–27 (4)
Lake area (km^{-2})	0.20–40 (2.0)	1.2–40 (1.70)	2.9–11.4 (1.49)
Mean depth (m)	1–70 (10)	14–70 (23)	1–20 (8)

TABLE 3. Temporal trends in planktonic biomass distribution in two lakes in 1987. The median size is the approximate size of an organism (μm ESD) at the 50% point in the biomass distribution, % at 80 μm is the proportion of total biomass which is due to organisms smaller than 80 μm, and total is concentration of the total biomass (ppm).

	Day of the year	Median size	% at 80 μm	Total
Lake Magog	128	8	97	3 477
	181	14	97	18 072
	193	28	74	18 328
	223	50	58	18 854
Lake Orford	128	4	98	2 086
	177	28	92	6 958
	199	10	93	6 466
	203	18	94	8 285
	237	40	65	20 140

methodological. The discrepancy is primarily due to the higher biomasses we observed in the 25–125 μm size range. Because we used a sampling tube that had a diameter 32 times larger to sample to a deeper depth (8 as opposed to 4 m) than did Sprules et al. (1983), we must sometimes have sampled deep chlorophyll peaks which are common in the area's lakes (M. Hanna, Canada Centre for Inland Waters, Burlington, Ont., unpubl. data). Perhaps, such peaks contain enough larger algae to account for the observed discrepancy, but if this were so, size spectra would be remarkably sensitive to sampling depth. Unfortunately, the basis of the differences in the distributions will remain speculation until parallel samples from both sampling protocols in both environments have been compared.

Kolmogoroff–Smirnoff tests showed that 46 of all 59 size distributions, and 17 of the 25 distributions with good coverage, did not differ significantly ($P < 0.05$) from a unimodal lognormal distribution (Fig. 3). Modes ranged from 19 to 75 μm ESD, with a median value of 48 μm for the 17 samples with both good coverage of the size range and significant fits (Table 4). A unimodal distribution appears to provide a first approximation of the plankton community size distribution, apparently because the phytoplankton mode dominates the communities we sampled.

Bimodal and trimodal distributions met with limited success (Table 5): only 7 of 25 samples with good coverage did not differ significantly from a mixture of two normal distributions, and only 10 samples did not differ significantly from trimodal distributions. The poor success rate of bimodal and trimodal fits is not entirely due to the underlying structure of the community, but also reflects weaknesses in the analytical approach and the data base. Even when the parameters cannot be estimated analytically because of the dominance of the phytoplankton mode, two or three modes are sometimes evident in the distribution (Fig. 3A). One problem is that the iterative algorithms used for estimation could have identified a "dead-end" in the parameter space which was not the optimal solution. A seemingly more pervasive problem lies in the number of parameters needed to estimate more complicated models. Bi- and trimodal models invoke five to eight parameters to describe only 39 points. Such fits require either more data points (i.e. size intervals) or more "perfect" data. Unfortunately, the use of narrower, and hence more numerous, intervals would not address the problem because the variability of sampling and sizing very narrow size ranges would generate a great deal of noise. More complicated models could be fit if the data were more or less arbitrarily divided into separate classes and each class fit to a unimodal distribution, but we were unwilling to impose our preconceptions on the data in this analysis.

Normalized Spectrum

Normalized spectra (Sprules and Munawar 1986) for the 25 samples with good coverage were computed using base 2 logarithms on both axes (Table 6; Fig. 4). Intercepts varied about sevenfold, with a mean of $6.4 \times 10^7 \cdot L^{-1}$ (95% CI of mean =

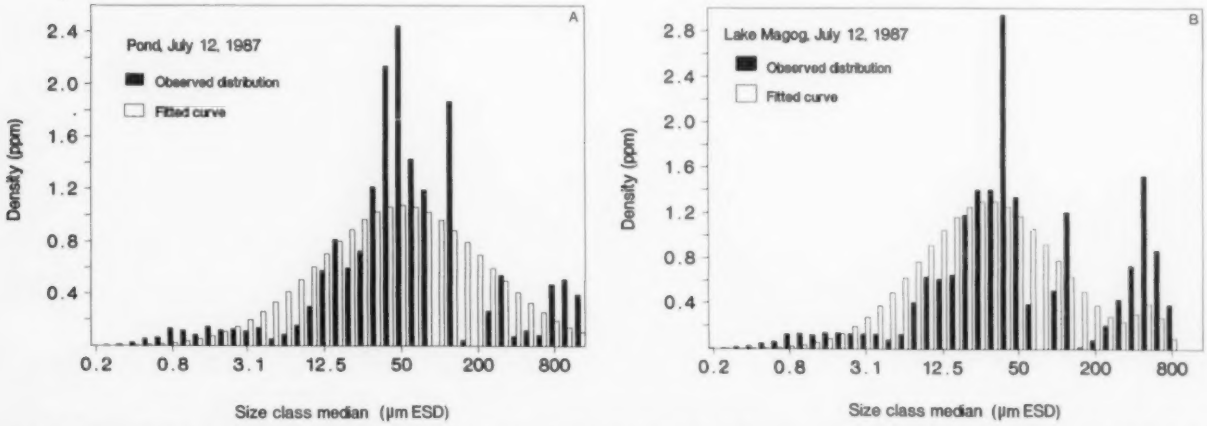

FIG. 3. Examples of (A) unimodal and (B) bimodal fits to the volume distribution. Note the apparent third mode below about 3 μm ESD in both panels.

TABLE 4. Parameters of all unimodal distributions successfully fitted to samples with good coverage of the size range, listed in order of decreasing total phosphorus concentration. The mean is the mean base 2 logarithm of organism volume (μm^3). The volume in a given size interval (on a base 2 log scale) may be estimated by multiplying the corresponding normal density $((2\pi\sigma^2)^{-1/2} \exp[-1/2((x-\mu)/\sigma)^2])$ by the total volume.

Lake	Day of the year	Mean (μ)	SD (σ)	Total volume (ppm)
Waterloo	229	16.45	4.20	192.7
Magog	223	15.77	6.56	29.5
Magog	193	15.92	6.93	18.3
Pond	193	15.96	6.48	17.3
Newport	204	17.04	7.13	14.7
South	235	15.49	5.40	29.9
Cerises	236	12.45	5.31	12.0
Central	228	17.53	7.64	35.9
Central	197a	17.66	8.22	19.6
Central	197b	17.78	8.13	19.4
North	226	12.51	5.18	9.4
Stukely	193	15.83	7.29	6.9
Orford	199	12.58	6.21	6.4
Lyster	227	13.62	5.43	11.9
Orford	203	11.82	4.31	8.3
Stukely	230	12.90	5.20	11.3
Orford	237	13.62	3.89	19.5

5.6×10^7 to 7.9×10^7). Since all of the regression slopes were highly significant ($P < 0.001$), there is indeed a consistent trend in biomass distribution over logarithmic size classes. The slopes varied from -0.75 to -1.01, with a mean of -0.89 (95% CI of mean = -0.87 to -0.91). Because the slope is significantly shallower ($P < 0.001$) than the slope of a spectrum with approximately equal biomass per class, the distribution is not uniform, but shows a small increase in biomass between successive size classes. The mean slope corresponds to a spectrum in which biomass tends to increase by about 7% between successive base 2 logarithmic size classes.

Over the range of sizes from 10^{-6} to 10^3 μg, the approximate median parameters (6 and -0.9) generated an integrated biomass estimate of 4.4 $mg \cdot L^{-1}$, which is well below the observed median (14.1 $mg \cdot L^{-1}$). Observed biomasses varied from 5.2 to 192 $mg \cdot L^{-1}$ whereas integrated estimates varied from 0.9 to 172 $mg \cdot L^{-1}$.

Integrated biomasses may tend to underestimate observation because the elevation of the regressions is typically depressed by relatively low numbers in extremely small and large size classes. This underestimation is particularly strong when biomasses are low.

The integrated estimates were significantly correlated ($P < 0.001$, $r^2 = 0.53$) with the observed total biomasses, where all values were log transformed (base 10) to stabilize variance (Fig. 5). This regression establishes that the spectrum may be used as a basis for comparison of samples, but the parameters of the relationship (log(obs) = $-0.87 + 0.48$ log(pred)) differed significantly from 0 and 1 and the scatter is high, so the strength of such a comparison is limited.

Sprules and Munawar (1986) reported normalized spectra for several Ontario lakes (Table 7). Their median slope values from replicated observations on individual lakes tended to be closer to -1 than ours and their median intercepts were smaller. Those authors did not provide sufficient information to allow a parametric comparison, but numeric integration of their curves over the same range as ours yielded values in the range 0.14–2.5 $mg \cdot L^{-1}$, compared with 0.8–172 $mg \cdot L^{-1}$ (median = 7.6) for our integrated totals. Our larger range results in part because each spectrum is based on a single sample, so extremes are not obscured by averaging, and because some of our samples had much higher phosphorus concentrations than those of Sprules and Munawar (1986). Nevertheless, our normalized spectra usually indicate higher total plankton biovolumes than do theirs, reflecting the larger populations of large phytoplankton in our samples.

The shallower slopes in our analyses also reflect the low biomasses of the smallest size classes we enumerated (which were not previously studied). Since the numbers in these classes are reduced, relative to expectations from Sheldon's hypothesis, the inclusion of bacterial size classes will rotate the regression counterclockwise, producing shallower slopes than do otherwise similar spectra. Thus the slopes of the normalized spectra will be independent of the size range enumerated only if Sheldon's hypothesis holds.

Variability of Parameters

The variabiilty of each of the parameters among the spectra was tested with Tukey's LSD test. This test showed no significant differences whatsoever among slopes or among intercepts of the normalized spectra. When Tukey's test was applied to the unimodal fits, four overlapping homogeneous sets of means were identified: 1–7, 5–10, 6–12, and 8–17 (where the numbers reflect the sequential order of the unimodal means from lowest to highest). Nevertheless, when the mean squared errors (variance estimates) of the 25 best spectra were compared, Bartlett's test showed significant variability ($P < 0.005$) among the residuals. The low variability of the parameters of these two models reflects both an underlying similarity in the average trends of the various size spectra (Fig. 4) and the insensitivity of the statistical models to departures from these averages among individual size classes.

The insensitivity of the models presents a problem for spectral comparisons and for the development of models to predict parameter differences among lakes. Size distributions from different samplings vary significantly, but these differences are lost in the simple statistical descriptions. More fruitful comparisons might focus on the departures from a general model rather than on the parameters derived from a series of specific models (e.g. Peters 1983a; Mazumder et al. 1988). Unfortunately, this approach will likely require large numbers of samples to distinguish sampling error from real departures.

Predictions

Because microscopic determinations of size spectra require considerable effort, a common goal in allometric ecology is the development of relations to permit simpler and more rapid estimation of the size structure of the community (Peters 1983a; Sprules and Munawar 1986). This section therefore develops correlations between the parameters of size distributions and other lake characteristics, using data collected in 1987, and where possible tests the predictive power of these relations with data collected in 1986.

Table 8 shows that the most effective predictors were aspects of lake trophic state: total phosphorus concentration, chlorophyll concentration, and seston dry weight, variables which are themselves correlated. Lake area and mean depth were only significant in the rather complex regressions describing the

TABLE 5. Parameters of bimodal and trimodal curves fitted to the distributions of plankton biovolume, listed in order of decreasing total phosphorus concentration. Units are base 2 logarithms of volume (μm^3). The proportion of each mode in a given interval is essentially as in the unimodal case (Table 4), but to determine the biovolume in any interval, the contributions of each mode must be weighted by the proportion of the total biovolume in that mode (Prop) and summed.

		Bimodal					
		First mode			Second mode		
Lake	Day of the year	Mean	SD	Prop	Mean	SD	Prop
Magog	193	13.5	5.39	0.81	26.1	1.04	0.19
Pond	224	17.2	7.47	0.46	22.5	0.98	0.54
South	235	7.4	8.21	0.08	16.2	4.41	0.92
Cerises	236	12.5	5.19	0.01	18.8	2.47	0.99
North	226	7.1	4.57	0.22	14.3	3.23	0.78
Baldwin	225	11.1	5.81	0.61	28.3	0.88	0.39
Orford	203	8.8	6.85	0.25	12.8	2.28	0.75

		Trimodal								
		First mode			Second mode			Third mode		
Lake	Day of the year	Mean	SD	Prop	Mean	SD	Prop	Mean	SD	Prop
Magog	193	2.7	6.29	0.13	14.3	2.51	0.63	26.0	1.30	0.24
Pond	193	6.0	8.71	0.13	15.8	2.65	0.71	28.6	3.18	0.16
Newport	204	1.9	2.27	0.11	13.9	4.74	0.53	20.4	1.00	0.36
Brome	144	3.1	3.41	0.49	11.1	1.00	0.29	21.7	2.75	0.22
South	186	−1.2	1.00	0.33	15.1	1.00	0.14	25.4	2.19	0.53
Baldwin	225	2.5	3.20	0.07	13.2	2.37	0.41	29.0	1.11	0.52
North	226	−4.3	1.59	0.02	11.5	3.55	0.51	15.2	1.00	0.47
Central	130	1.7	3.76	0.51	12.8	5.58	0.30	15.3	1.64	0.19
Lyster	227	9.1	5.18	0.25	14.2	1.99	0.66	27.2	1.00	0.09
Orford	203	2.2	2.65	0.09	12.7	2.63	0.90	25.5	1.32	0.01
Stukely	230	3.8	6.13	0.14	12.3	2.23	0.76	22.1	1.00	1.12
Orford	199	6.0	4.25	0.19	13.0	1.95	0.66	28.9	1.12	0.15

parameters of the unimodal fits to the data. In general, the proportion of the variance explained by the relations in Table 8 is lower than those reported in broader studies of lake trophic response (Peters 1986; Seip and Ibrekk 1988), but this reflects the more limited range of phosphorus concentrations available to us. The uncertainty of the predictions, as reflected in the standard errors listed in Table 8, is of the same order as those reported in other general models of lake response.

The mean and standard deviations of the unimodal, lognormal description of the biomass distributions were best correlated with a combination of the logarithms of the concentrations of total phosphorus, sestonic dry weight, and mean depth. Each variable was similarly important to the regression, but collinearities between the two dependent variables ($r = 0.57$) and between the first two independent variables ($r = 0.54$) and the small size of the data set make interpretation of the multiple regressions problematical. Bivariate regression suggested no escape from this complexity because no single predictor variable was significantly correlated with either parameter.

Total epilimnetic phosphorus concentration proved to be the most useful simple correlate of the parameters of normalized spectra. As total phosphorus increases, the slope becomes less negative or shallower, indicating that larger organisms increase disproportionately. The intercept of the normalized spectrum, representing the biomass at 1 μm^3, also increases with total phosphorus, reflecting a tendency for biomass to increase in all size classes as phosphorus concentration rises. These patterns are consistent with general trends in the phytoplankton established previously by literature surveys (Watson and Kalff 1981; Watson and McCauley 1988) and size structure analyses (Peters 1983a; Sprules and Munawar 1986).

The numerical abundance of the plankton was very highly correlated with size, for 94% of the base 2 log of the numerical density in a size interval was explained by a regression on the log of mean size in that interval (Table 8). This model, based on 1987 data, explained 82% of the variation in the independent 1986 abundance data. One variable, log total phosphorus improved this model significantly, but only slightly increased the variation explained in the original data. Nevertheless, this augmented model explained 87% of the variation in the independent data set.

As one might anticipate from previous work (Peters 1986; Seip and Ibrekk 1988), the logarithm of total phosphorus explained much of the variation in the logarithm of biovolume; this relationship was not significantly improved by the inclusion of other likely regressors (e.g. chlorophyll, lake morphometry) in the model. When the regression in Table 8 was applied to the 1986 data, predictions and observations were significantly correlated. When a similar regression between total volume and total phosphorus was fitted to the 1986 data, the mean squared errors of the two models were sufficiently similar (Bartlett's test, $P < 0.25$) to allow a direct comparison of the two parameter sets. An F-test showed that the two regressions did not differ significantly (Fig. 6).

Phosphorus as an Index of Biomass

Previous work in Lake Memphremagog (Peters 1983a) showed that the particulate phosphorus concentration summed

TABLE 6. Parameters of the normalized spectra for samples with good coverage of the size range, arranged in order of declining intercept. The independent variable is the base 2 logarithm of organism volume (μm^3). The dependent variable is the base 2 logarithm of the normalized density ($\times 10^{-6} \cdot L^{-1}$).

Lake	Day of the year	Intercept	SE	Slope	SE	F	R^2	SEE
Waterloo	229	7.981	0.430	−0.752	0.033	516	0.94	1.90
Lovering	177	7.066	0.910	−1.002	0.069	214	0.88	3.91
South	235	6.679	0.734	−0.951	0.048	387	0.92	3.27
South	186	6.499	0.532	−0.829	0.036	520	0.94	2.36
Central	228	6.327	0.458	−0.870	0.031	783	0.96	2.03
O'Malley	177	6.304	0.734	−0.797	0.065	152	0.84	3.22
Cerises	236	6.272	0.521	−0.904	0.043	443	0.94	2.29
Magog	193	6.247	0.516	−0.877	0.035	618	0.95	2.29
Pond	193	6.216	0.578	−0.915	0.037	622	0.94	2.58
Central	197	6.079	0.425	−0.867	0.029	903	0.97	1.89
Magog	223	6.068	0.384	−0.853	0.027	1011	0.97	1.70
Pond	224	6.020	0.520	−0.872	0.034	649	0.95	2.31
Central	197	5.960	0.429	−0.862	0.029	878	0.97	1.90
Central	166	5.957	0.533	−0.764	0.047	265	0.90	2.34
Cerises	193	5.952	0.532	−0.760	0.047	263	0.90	2.33
North	226	5.870	0.588	−0.938	0.045	441	0.93	2.60
Baldwin	225	5.827	0.599	−0.944	0.040	552	0.94	2.67
Stukely	230	5.700	0.655	−0.942	0.047	410	0.92	2.90
Central	186	5.636	0.703	−0.894	0.057	250	0.89	3.09
Lyster	227	5.608	0.734	−0.941	0.052	326	0.91	3.25
Orford	237	5.542	0.650	−0.837	0.055	236	0.89	2.86
Orford	203	5.483	0.800	−1.005	0.057	312	0.90	3.54
Newport	204	5.416	0.460	−0.866	0.033	682	0.95	2.04
Orford	199	5.373	0.627	−0.963	0.044	483	0.94	2.78
Stukely	193	5.162	0.683	−0.975	0.043	504	0.93	3.05

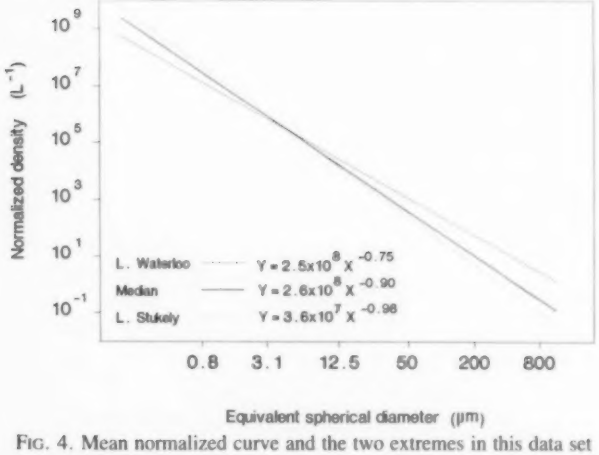

FIG. 4. Mean normalized curve and the two extremes in this data set (n = 25).

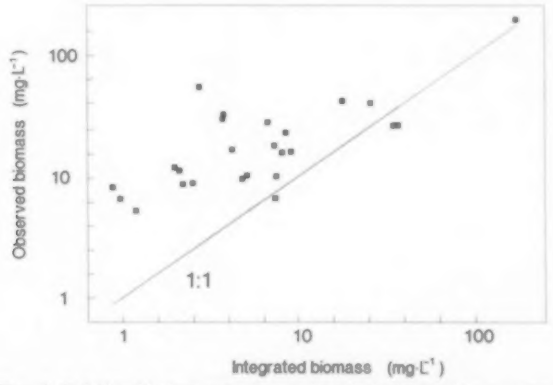

FIG. 5. Relationship between observed total planktonic biomasses and the estimates obtained from integration of the normalized curves fitted to these data.

over successive logarithmic size classes increased linearly over the range from 1 to 100 μm ESD. Such linear relationships indicate that the phosphorus content per size class is constant within a given sample. Peters (1983a) interpreted this to mean that biomass was also unaffected by size, although this interpretation rests on the untested assumptions that the ratio of phosphorus to biomass does not change with either size or total phosphorus concentration.

To test those assumptions, we conducted a two-way analysis of variance of the log-transformed mean phosphorus/biomass ratios for three size classes (0.4–5, 5–80, and >80 μm) in four ranges of total phosphorus concentrations (<9, 9–21, 21–52, and >52 $mg \cdot m^{-3}$). These intervals correspond to intervals of equal range in log-transformed phosphorus concentration values. The model explained 71% of the variation in the data (n = 121, F = 39.2, $P < 0.001$, SEE = 0.1584). The predicted mean values (Table 9) show a trend to decreasing ratios with increasing size ($P < 0.01$) and perhaps to increasing ratios with increasing total phosphorus concentration ($P > 0.05$; not significant). The mean values in the table suggest that the phosphorus concentration in picoplankton is about eight times higher than that in netplankton. Assuming that dry weight is about 30% of wet weight (Peters and Downing 1984), the mean phosphorus content of algae from Vinogradov's (1953) data (0.69% of dry mass) converts to a wet phosphorus/biomass ratio of 0.0023, a value intermediate between those of the small and

TABLE 7. Parameters (range and median) of normalized spectra for Ontario lakes computed by Sprules and Munawar (1986). For purposes of comparison, the values for our spectra computed over a similar size range (n = 25) are included, after conversion to base 10 logarithms and expressing size in μg fresh weight.

Lake	Slope	Intercept
Inland lakes	−0.92 to −1.05 (−0.98)	0.92 to 1.93 (1.36)
St. Clair	−0.76 to −1.05 (−0.90)	1.11 to 1.83 (1.46)
Erie	−0.77 to −1.24 (−0.99)	1.79 to 2.41 (1.99)
Ontario	−0.90 to −1.04 (−0.97)	1.35 to 2.16 (1.82)
Huron	−0.90 to −1.18 (−1.02)	0.64 to 1.65 (0.90)
Superior	−1.00 to −1.15 (−1.10)	0.41 to 0.73 (0.61)
This study	−0.75 to −1.01 (−0.90)	1.70 to 3.89 (2.41)

medium size classes in our analysis. Several authors reported that phosphorus concentrations in different algal species decrease as cell size increases (Shuter 1978; Smith and Kalff 1982; Vézina 1986) and solution of their relations results in phosphorus/biomass ratios which are consistent with the values in Table 9. Peters (1983a) himself noted a trend to lower phosphorus concentrations with increasing organism size in Vinogradov's (1953) data, and our analysis shows that a similar trend exists among whole size classes in nature.

The size dependence of the phosphorus/biomass ratio has important implications for the use of phosphorus as an index of biomass. First, the phosphorus contents of different size classes will be more variable than their biomasses. Second, the high phosphorus concentration in the smallest size classes will lead to overestimates of small organism biomass compared with that of larger organisms. Hence, the linear trends in the relationship between cumulative phosphorus and organism size reported by Peters (1983a) actually indicate that biomass increases with size. This confirms the tendency indicated by our results from the normalized spectrum.

Extrapolations

If the mean slope and intercept of the normalized spectrum are used to predict the abundance of organisms at larger sizes, the projected 7% increase in biomass per log 2 size class is necessarily constrained by the more rapid increase in organism size (doubling between classes). For example, if the largest organism is assumed to be large lake sturgeon, then the largest size interval spans the range 18–36 kg (geometric mean class weight = 25 kg), and the mean parameters from the normalized spectrum imply a total abundance of about 150 000 organisms·km^{-3}. This seems unreasonable, even though the 95% confidence interval is very broad (between 12 000 and 1 880 000), so we suspect that biomass per size class does not peak in the largest class, but declines in the largest size classes, as our data show it to do in extremely small ones. This would be consistent with the findings of Minns et al. (1987) who described the whole biomass spectrum of the eutrophied Bay of Quinte as a unimodal distribution.

This study adds to the growing evidence that the concept of even distributions of biomass across logarithmic size classes is only a first approximation: the biomasses of logarithmically increasing size classes are usually within the same order of magnitude. It would be somewhat more correct to see biomass distributions as hierarchical summations in which a single, general, roughly log-normal, unimodal distribution is itself composed of several to many smaller unimodal distributions. The frequent tendency for relatively and absolutely small organisms to occur at low biomass, which is implicit in unimodal distributions, may reflect a combination of reduced levels of performance in such animals (Banse 1976), lower levels of predation (Banse and Mosher 1980), and effectively oligotrophic conditions imposed by the larger and more effective competitors that comprise the mode of the distribution (Peters 1991). The symmetry of the distributions and the suggestion that organisms in the upper tail also exhibit relatively low levels of performance (Dickie et al. 1987a) hint that similar processes — effectively low resource levels and competition from the mode — may inhibit both extremes of unimodal size distributions. However, the frequency of exceptions to these patterns suggests that the distributions are transient and dynamic as forces both inside and outside the size classes of interest shift the ecological advantage among size classes. Parametric size distributions are at best averaged abstractions, and departures from such idealized distributions must be expected when communities and subcommunities are examined over restricted temporal and spatial scales (Hobson 1988).

TABLE 8. Predictive regressions for planktonic size distributions where TVOL = volume density of plankton (μm^3 × 10^{-6}·L^{-1}), TP = total epilimnetic phosphorus concentration (mg·m^{-3}), DW = epilimnetic dry mass concentration (μg·L^{-1}), Z = mean depth (m), N = numeric density of plankton in a size interval (× 10^{-6}·L^{-1}), SIZE = volume of a single organism (μm^3), UNIMODE = mean of normal distribution of volume over $\log_2$ sizes, UNISD = standard deviation of this normal distribution, NSLOPE = slope of the normalized spectrum, and NINT = intercept of the normalized spectrum. *$0.05 > P > 0.01$, **$0.01 > P$.

	Equation	n	SE_{est}	R^2	F
$\log_{10}$ TVOL	= 3.3 + 0.85 $\log_{10}$ TP	25	0.237	0.55	28**
UNIMDE	= 6.4 + 6.9 $\log_{10}$ TP − 4.4 $\log_{10}$ DW + 2.6 $\log_{10}$ Z	17	1.22	0.69	9**
UNISD	= 2.4 + 3.1 $\log_{10}$ TP − 3.6 $\log_{10}$ DW + 1.7 $\log_{10}$ Z	17	0.85	0.67	8**
NSLOPE	= −1.04 + 0.13 $\log_{10}$ TP	25	0.062	0.28	9**
NINT	= 4.30 + 1.50 $\log_{10}$ TP	25	0.401	0.56	10**
$\log_2 N$	= 5.756 − 0.793 $\log_2$ SIZE	1647	1.900	0.94	24 624**
$\log_2 N$	= 3.601 − 0.792 $\log_2$ SIZE + 0.533 $\log_2$ TP	1647	1.834	0.94	13 266**

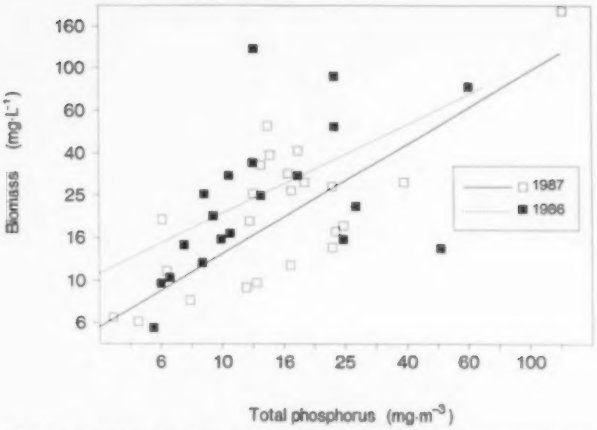

FIG. 6. Relationship between observed total plankton biomass and total phosphorus concentration in 1986 and 1987. There is no significant difference between the two independently fitted relationships.

TABLE 9. Results of the two-way analysis of variance of the particulate phosphorus/biomass ratio. Columns represent three size classes, and rows represent four levels of total phosphorus concentration. The four values in each cell are the number of observations, the mean predicted $\log_{10}$ ratio, the standard error of this estimate, and the back-transformed ratio corrected for transformation bias (Sprugel 1983).

	0.4–5 μm	5–80 μm	>80 μm
TP < 9	8	7	5
	−2.77	−3.63	−3.68
	0.0992	0.1020	0.1140
	0.0026	0.0004	0.0003
9 < TP < 21	35	24	11
	−2.63	−3.49	−3.54
	0.0599	0.0693	0.0925
	0.0036	0.0005	0.0004
21 < TP < 52	12	9	5
	−2.45	−3.30	−3.36
	0.0873	0.0927	0.1090
	0.054	0.0008	0.0007
TP > 52	3	1	1
	−2.39	−3.25	−3.30
	0.1800	0.1880	0.1940
	0.0062	0.0009	0.0008

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References

AHRENS, M. A. 1989. The size distribution of the limnoplankton. Ph.D. thesis, McGill University, Montreal, Que. 190 p.

AHRENS, M. A., AND R. H. PETERS. 1991. Plankton community respiration: relationships with size distribution and lake trophy. Hydrobiologia. (In press)

BAILEY-WATTS, A. E. 1986. Seasonal variation in size spectra of phytoplankton assemblages in Loch Leven, Scotland. Hydrobiologia 138: 25–42.

BANSE, K. 1976. Rates of growth, respiration, and photosynthesis of unicellular algae as related to cell size — a review. J. Phycol. 12: 135–140.

BANSE, K., AND S. MOSHER. 1980. Adult body mass and annual production/biomass relationships of field populations. Ecol. Monogr. 50: 355–379.

BORGMANN, U. 1982. Particle-size conversion efficiency and total animal production in pelagic ecosystems. Can. J. Fish. Aquat. Sci. 39: 668–674.

1987. Models on the slope of, and biomass flow up, the biomass size spectrum. Can. J. Fish. Aquat. Sci. 44: 136–140.

CLARKE, K. R. 1984. Parameter estimation for mixture distributions of species sizes, appendix p. 40–41. *In* R. M. Warwick. species size distributions in marine benthic communities. Oecologia (Berl.) 61: 32–41.

DICKIE, L. M., S. R. KERR, AND P. R. BOUDREAU. 1987a. Size dependent processes underlying regularities in ecosystem structure. Ecol. Monogr. 57: 233–250.

DICKIE, L. M., S. R. KERR, AND P. SCHWINGHAMER. 1987b. An ecological approach to fisheries assessment. Can. J. Fish. Aquat. Sci. 44(Suppl. 2): 68–74.

DRAPER, N. R., AND H. SMITH. 1981. Applied regression analysis. 2nd ed. John Wiley and Sons, New York, NY. 709 p.

ECHEVARRIA, F. P. CARRILLO, F. JIMINEZ, P. SANCHEZ-CASTILLO, L. CRUZ-PIZARRO, AND J. RODRIGUEZ. 1990. The size abundance distribution and taxonomic composition of plankton in an oligotrophic, high mountain lake (La Caldera, Sierra Nevada, Spain). J. Plankton Res. 12: 415–422.

GRIESBACH, S., R. H. PETERS, AND S. YOUAKIM. 1982. An allometric model for pesticide bioaccumulation. Can. J. Fish. Aquat. Sci. 39: 727–735.

HANSON, J. M., E. E. PREPAS, AND W. C. MACKAY. 1989. Size distribution of the macroinvertebrate community in a freshwater lake. Can. J. Fish. Aquat. Sci. 46: 1510–1519.

HOBBIE, J. E., R. J. DALEY, AND S. JASPERS. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 1225–1228.

HOBSON, L. A. 1988. Volume–frequency distributions of marine phytoplankton in a temperate neritic environment. Mar. Ecol. Prog. Ser. 48: 47–55.

JOHN, P. W. M. 1971. Statistical design and analysis of experiments. Mcmillan, New York, NY. 356 p.

LAL, D. 1977. The oceanic microcosm of particles. Science (Wash., DC) 198: 997–1009.

LUND, J. W., C. KIPLING, AND E. D. LECREN. 1958. The inverted microscope method of estimating algal cell numbers and the statistical basis of estimation by counting. Hydrobiologia 11: 143–170.

MAZUMDER, A., D. J. MCQUEEN, W. D. TAYLOR, AND D. R. S. LEAN. 1988. Effects of fertilization and planktivorous fish (yellow perch) predation on size distribution of particulate phosphorus and assimilated phosphate: large enclosure experiments. Limnol. Oceanogr. 33: 421–430.

MENZEL, D. W., AND N. CORWIN. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. Limnol. Oceanogr. 10: 280–282.

MINNS, C. K., E. S. MILLARD, J. M. COOLEY, M. G. JOHNSON, D. A. HURLEY, K. H. NICHOLLS, G. W. ROBINSON, G. E. OWEN, AND A. CROWDER. 1987. Production and biomass size spectra in the Bay of Quinte, a eutrophic ecosystem. Can. J. Fish. Aquat. Sci. 44 (Suppl. 2): 148–155.

PACE, M. L. 1986. An empirical analysis of zooplankton community size structure across lake trophic gradients. Limnol. Oceanogr. 31: 45–55.

PETERS, R. H. 1983a. Size structure of the plankton community along the trophic gradient of Lake Memphremagog. Can. J. Fish. Aquat. Sci. 40: 1770–1778.

1983b. The ecological implications of body size. Cambridge University Press, Cambridge. 329 p.

1985. Seasonal and trophic effects on size structure of the planktonic communities in four lakes of northern Italy. Mem. Ist. Ital. Idrobiol. 43: 91–104.

1986. The role of prediction in limnology. Limnol. Oceanogr. 31: 1143–1159.

1991. Lessons from the size efficiency hypothesis. I. The general refuge concept. Sel. Monogr. Unione Zool. Ital. (In press)

PETERS, R. H., AND J. A. DOWNING. 1984. Empirical analysis of zooplankton filtering and feeding rates. Limnol. Oceanogr. 29: 763–784.

PLATT, T., AND K. DENMAN. 1978. The structure of pelagic marine ecosystems. Rapp. P.-v. Réun. Cons. int. Explor. Mer 173: 60–65.

PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25: 943–948.

ROBINSON, W. R., R. H. PETERS, AND J. ZIMMERMANN. 1983. The effects of body size and temperature on metabolic rate of organisms. Can. J. Zool. 61: 281–288.

RODRIGUEZ, J., AND M. M. MULLIN. 1986. Relation between biomass and body weight of plankton in a steady state oceanic ecosystem. Limnol. Oceanogr. 31: 361–370.

SCHWINGHAMER, P. 1981. Characteristic size distributions of integral benthic communities. Can. J. Fish. Aquat. Sci. 38: 1255–1269.

1983. Generating ecological hypotheses from biomass spectra using causal analysis: a benthic example. Mar. Ecol. Prog. Ser. 13: 151–166.

SEBER, G. A. F. 1977. Linear regression analysis. John Wiley and Sons, New York, NY. 465 p.

SEIP, K. L., AND H. IBREKK. 1988. Regression equations for lake management. Verh. Int. Ver. Limnol. 23: 778–85.

SHELDON, R. W., A. PRAKASH, AND W. H. SUTCLIFFE. 1972. The size distribution of particles in the ocean. Limnol. Oceangr. 17: 327–340.

SHUTER, B. J. 1978. Size dependence of phosphorus and nitrogen subsistence quotas in unicellular microorganisms. Limnol. Oceanogr. 23: 1248–1255.

SMITH, R. E., AND J. KALFF. 1982. Size dependent phosphorus uptake kinetics and cell quota in phytoplankton. J. Phycol. 18: 275–284.

SOMMER, U., Z. M. GLIWICZ, W. LAMPERT, AND A. DUNCAN. 1986. The PEG model of seasonal succession of planktonic events in fresh waters. Arch. Hydrobiol. 106: 433–471.

SPRUGEL, D. G. 1983. Correcting for bias in log transformed allometric equations. Ecology 64: 209–210.

SPRULES, W. G., J. M. CASSELMAN, AND B. J. SHUTER. 1983. Size distribution of pelagic particles in lakes. Can. J. Fish. Aquat. Sci. 40: 1761–1769.

SPRULES, W. G., AND R. KNOECHEL. 1984. Lake ecosystem dynamics based on functional representations of trophic components, p. 383–403. *In* D. G. Meyers and J. R. Strickler [ed.] Trophic interactions within aquatic ecosystems. AAS Symposium 85, Washington, DC.

SPRULES, W. G., AND M. MUNAWAR. 1986. Plankton size spectra in relation to ecosystem productivity, size, and perturbation. Can. J. Fish. Aquat. Sci. 43: 1789–1794.

STRASKRABA, M. 1980. The effects of physical variables on freshwater production: analysis based on models, p. 13–84. *In* E. D. LeCren and R. H. McConnell [ed.] The functioning of freshwater ecosystems. Cambridge University Press, Cambridge.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. 2nd ed. Bull. Fish. Res. Board Can. 167.

VÉZINA, A. F. 1986. Body size and mass flow in freshwater plankton: models and tests. J. Plankton Res. 8: 939–956.

VINOGRADOV, A. P. 1953. The elementary chemical composition of marine organisms. Mem. No. 2, Sears Foundation, New Haven, CT.

WARWICK, R. M., AND I. R. JOINT. 1987. The size distribution of organisms in the Celtic Sea: from bacteria to Metazoa. Oecologia 73: 185–191.

WATSON, S., AND J. KALFF. 1981. Relationships between nannoplankton and lake trophic status. Can. J. Fish. Aquat. Sci. 38: 960–967.

WATSON, S., AND E. MCCAULEY. 1988. Contrasting patterns of net and nanoplankton production and biomass among lakes. Can. J. Fish. Aquat. Sci. 45: 915–920.

Effect of Sampling Protocol on Estimates of Phosphorus and Chlorophyll Concentrations in Lakes of Low to Moderate Trophic Status

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Hanna, M., and R. H. Peters. 1991. Effect of sampling protocol on estimates of phosphorus and chlorophyll concentrations in lakes of low to moderate trophic status. Can. J. Fish. Aquat. Sci. 48: 1979–1986.

Methods of sampling lake phosphorus and chlorophyll concentrations were assessed by comparing mean values for 16 lake basins in southern Quebec. Integrating tube and discrete-depth samples were analyzed to yield six different subsurface, depth-integrated, and volume-weighted average values representing epilimnetic, euphotic, and trophogenic zones. The different protocols were found to be similarly precise. Epilimnetic samples yield low values, while trophogenic samples yield high ones; however, the differences in protocol accounted for less than 50%. Analysis of variance associated most of the uncertainty in these estimates with lake and sampling date and showed that sampling site rarely had a significant effect. Year had only a minor effect, but interannual variation is probably underestimated, since these data represent only two years. These patterns suggest that representative sampling for a given lake requires only a single sample per visit but several visits within a season. Since chlorophyll varied more spatially than phosphorus, sampling protocol affects its estimates to a greater extent. Phosphorus–chlorophyll regressions based on data collected in different ways were very similar, so differences in protocol and sampling depth seem unlikely to contribute to the high uncertainty in existing phosphorus response models.

Les méthodes d'échantillonnage utilisées pour mesurer les concentrations de phosphore et de chlorophylle ont été évaluées en comparant les valeurs moyennes de 16 bassins lacustres du sud du Québec. Des échantillons ponctuels (surface et pondéré par volume) ainsi que des échantillons prélevés à l'aide de tubes intégrés (en profondeur) ont été analysés de sorte à produire six différentes mesures représentant la surface, l'épilimnion, la zone euphotique et la zone trophogène. Ces différents protocoles offrent le même degré de précision. Les échantillons de l'épilimnion présentent des concentrations plus faibles que ceux de la zone trophogène; les différences moyennes sont inférieures à 50 %. L'analyse de variance attribue la majeure partie de l'incertitude de ces estimations aux lacs et à la date d'échantillonnage, le site ayant rarement un effet significatif. L'année a un effet moindre, mais la variation inter-annuelle est probablement sous-estimée puisqu'elle n'est basée que sur deux ans. Ces tendances suggèrent qu'un échantillonnage représentatif d'un lac requiert un seul site par date mais plusieurs dates d'échantillonnage au cours de la saison. La chlorophylle étant plus variable dans l'espace que le phosphore, le protocole d'échantillonnage influencera davantage les mesures de chlorophylle. Les régressions phosphore–chlorophylle basées sur des données prélevées de différentes façons sont semblables, et donc des différences de protocole d'échantillonnage ne contribuent vraisemblablement pas à l'incertitude existant dans les modèles de prédictions de l'effet du phosphore.

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Mean concentrations of phosphorus and chlorophyll in lake waters are such useful indices of productivity and cultural eutrophication (OECD 1982; Peters 1986; Seip and Ibrekk 1988) that these properties are now routinely included in lake assessment and monitoring programs. In this role, both estimates must represent a lake as effectively and efficiently as possible (Thornton et al. 1982). Sampling protocols should minimize both the variance associated with the mean and the bias associated with spatial and temporal variations, thus yielding the most informative (or least uncertain) estimates for use as descriptors and as predictor variables in regressions and theories. The order-of-magnitude error associated with existing phosphorus response models (Dillon and Rigler 1974; Peters 1986; Benndorf 1988) suggests that bias and uncertainty may be significant and that attention to the details of sampling and analysis might improve existing relationships.

One element in such a sampling program is the selection of appropriate sampling sites, depths, and frequencies for the determination of general patterns in water quality. Common practices in comparative limnology suggest that these sources of error are often deemed of little importance. Researchers building nutrient response relationships with literature data assume that interannual variation is of the same magnitude as interlake variation (because different lake-years are taken as independent samples), that intersite variation is small (because most regressions depend on unreplicated samples collected at a single site near the deepest part of the lake; Peters 1989), that seasonal variation is adequately represented by only part of the growing season (because sampling is sometimes restricted to summer), and that all sampling methods and depths are com-

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parable (because these aspects are sometimes not reported and often mixed in larger scale regressions; Marshall and Peters 1989).

Only some of these traditions are justified by more formal analyses. Knowlton et al. (1984) examined the effects of sample replication at a single site and of seasonal and interannual variation at that site on the variance in estimates of mean phosphorus and chlorophyll. They concluded that replication error was negligible but that seasonal and annual variation contributed significantly to the observed variance. Marshall et al. (1988) examined the effect of sampling frequency on the variance associated with seasonal averages of chlorophyll concentration and showed that three to seven samples were needed to estimate the mean with reasonable precision and accuracy. Many regressions in "predictive limnology" are based on fewer samples. Neither paper considered the effect of sampling site, but for nutrients, this seems small (Hilton et al. 1989), except very close to shore (Peters and Cattaneo 1984; Cattaneo 1987). Finally, Hilton et al. (1989) found that nutrient levels in integrated tube samples from the trophogenic zone of eutrophic lakes exceeded nutrient levels measured at the surface.

These studies identify temporal and vertical variation as potentially important in sampling phosphorus or chlorophyll, but do not assess the effects of differences in sampling protocol at each visit. Moreover, none of these studies considered the effects of such sources of error in estimating chlorophyll and phosphorus concentrations as they are used in response models. This study addresses these problems by comparing bias and imprecision in estimates of total phosphorus and chlorophyll *a* concentrations derived from subsurface samples, depth-integrated tube samples, and volume-weighted samples of the upper strata for 16 different lake basins representing the range of trophic states common in southern Quebec and in much of the rest of eastern and northern Canada, but not hypertrophic or ultraoligotrophic waters.

Methods

Each of 16 lake basins (Table 1) in southern Quebec was visited a total of five to six times from July to mid-August of 1986 and 1987. Of these lakes, lac Hertel is perched on an intrusion into the St. Lawrence Lowlands at Beloeil, Quebec (45.5°N, 73°W), Lakes Achigan, Croche, and Echo lie in the Southern Laurentians (46°N, 74°W), and the remaining lakes are near Magog, Quebec (45°N, 72°W). Sampling was limited to midsummer to approximate the sampling regimes used in most seasonal studies (Marshall and Peters 1989) and to avoid the possibility of dramatic differences associated with different seasons and degrees of stratification. Two deep sites were selected in each basin as permanent sampling stations, with the exception of the southern basin of Lake Memphremagog where only one site was sampled.

Because we were concerned that vertical structure in the physical environment affects measurements, sampling at each site began with the determination of the Secchi disc depth and the depth profiles of temperature (Leeds and Northrup model 7932), light (Kahl Scientific underwater irradiameter model 268WA310), and turbidity (Philipp Schenk transparency meter). Epilimnion depth was estimated as the depth where the thermal gradient reaches 1°C/m (Welch 1948). In the few cases where double epilimnia were obvious, the deeper one was more pronounced and was used for calculations (Hanna 1990). The depths of the "trophogenic" and "euphotic" zones were estimated as twice the Secchi disc depth and as the depth of the 1% light level, respectively. Integrated samples were then collected by sampling to the depths of the epilimnion and the trophogenic zone using a 2.5-cm Tygon tube. Discrete samples were collected from subsurface to the deeper of 20 m or the depth at which light was no longer detectable, or to 1 m above the bottom using a 2-L van Dorn bottle. When the turbidometer indicated the presence of a deep turbidity peak, sampling was intensified and extended to fully define this peak. Samples were collected at 2-m intervals except in the thermocline and where the turbidometric profile indicated rapid changes in suspended material. In those regions, samples were collected at 1-m intervals.

TABLE 1. Characteristics of the 16 lake basins studied. SD, Secchi disc depth; Z_{EU}, depth of the euphotic zone defined as the depth where ambiant light is 1% of subsurface levels; Z_{EPI}, epilimnion depth. All values are means (n = 10–12), observed in the course of this study (midsummer 1986 and 1987). Z_{Mean}, mean lake depth; Z_{Max}, maximum lake depth; Area, lake area; MEL, maximum effective length of the lake.

Lake	SD (m)	Z_{EU} (m)	Z_{EPI} (m)	Z_{Mean} (m)	Z_{Max} (m)	Area (ha)	MEL (km)
Magog	1.8	4.2	7.7	9.8	19.2	1100	8.0
Echo	3.8	5.7	2.8	5.9	9.1	190	1.3
Hertel	3.1	6.2	3.1	3.8	8.3	13	0.32
Memphremagog (South)	4.4	7.5	6.3	6.2	13	5600	42.0
Memphremagog (Central)	5.1	10.2	10.5	44.3	104	2400	42.0
Massawippi	3.7	8.2	5.8	41.6	85.7	1800	10.0
Lovering	3.9	6.1	6.8	10.3	24.9	490	4.5
Memphremagog (North)	5.4	11.0	10.5	14.7	32	2000	42.0
Petit Brompton	4.4	7.0	4.4	7.8	19.5	73	1.4
Croche	3.9	5.9	2.4	4.5	11.5	5	0.42
Montjoie	4.0	6.4	4.5	7.9	22.2	340	3.1
Brompton	4.0	5.7	6.6	11.5	42	1100	7.2
Achigan	4.2	7.0	3.8	12.3	21	590	4.1
Orford	5.6	12.9	5.4	17.7	48	130	1.9
Stukley	4.5	9.0	5.6	13.6	32.2	410	3.1
Bowker	7.3	17.3	7	26	59	230	5.6

In the laboratory, total phosphorus concentrations were determined in duplicate with the ascorbic acid modification of the molybdenum blue procedure (Strickland and Parsons 1972) following persulphate digestion under pressure (Menzel and Corwin 1965). Duplicate chlorophyll concentrations were determined by collecting the seston on a GFC filter and then extracting the pigment in 95% ethanol; after centrifugation, the concentration of chlorophyll *a* in the supernatant was determined trichromatically (Bergmann and Peters 1980) without correction for phaeophytin. This simple method was chosen to simplify analysis in a program that involved more than 6000 determinations for each of phosphorus and chlorophyll. Marshall et al. (1988) found no significant effect of chlorophyll methodologies, including this simple ethanol extraction, on the temporal variance in chlorophyll estimations. Subsequent analysis used the means of these duplicate determinations.

These data were used to generate six classes of phosphorus and chlorophyll concentrations that might be thought to represent the lake (Fig. 1). One class was constituted by "subsurface" samples, taken just below the water surface with a van Dorn bottle. Two classes were "integrating tube" samples representing the epilimnion and the trophogenic zone. The final three classes were "composites" representing the volume-weighted average concentrations over different strata as

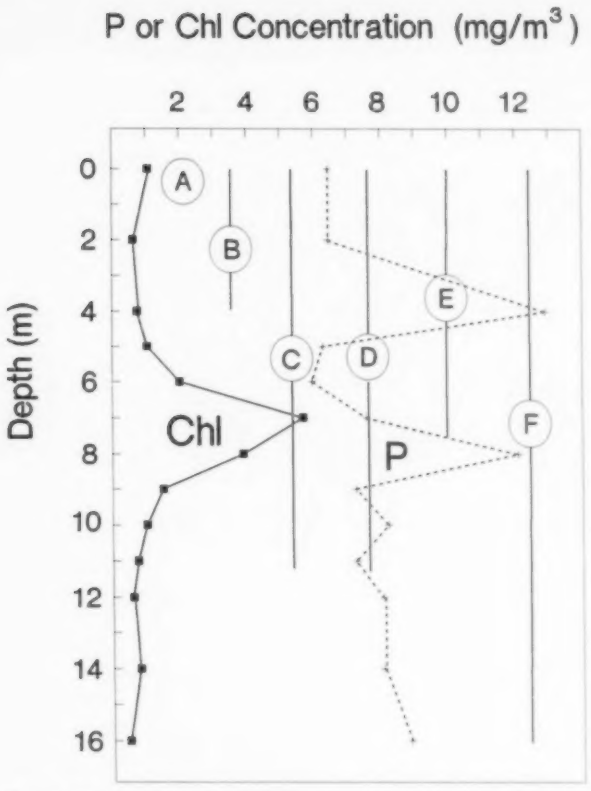

FIG. 1. Diagram of the six sampling protocols used in this study superimposed on plots of the vertical distribution of chlorophyll and total phosphorus observed at one site on Lac Achigan in July 1986 (transparency = 5.6 m; epilimnion depth = 4 m). Protocol A (Subsurf.) represents a simple subsurface grab with a van Dorn sampler; protocols B (Tube EPI) and C (Tube 2SD) represent integrated tube samples of the epilimnion and the trophogenic zone defined as twice the Secchi disc depth, respectively. The remaining protocols used composite average values, weighted by strata volumes, of van Dorn samples collected (D) in the trophogenic zone (Comp. 2SD), (E) in the euphotic zone defined as the 1% light level (Comp. 1%), and (F) from the deepest part of the column (Comp. Col.).

determined from the van Dorn samples. Two of these volume-weighted, composite samples represent the "euphotic zone" measured as the zone above the depth of 1% light level (Z_{EU}, metres) and the "trophogenic zone" measured as that above twice the Secchi disc depth (SD, metres). These two depths are similar in these lakes, but not identical ($Z_{EU} = 0.57 + 1.7$ SD; $r^2 = 0.59$; $n = 174$). The trophogenic zone is deeper than the euphotic zone (mean difference = 0.45 m) in 115 of 174 comparisons. The third composite was a "column" sample, representing the water column from the surface to the deepest sample taken, i.e. to 1 m above the bottom or to the deepest of the lower edge of any deep-water peak, the undetectable light level, or to 20 m.

In principle, the volume-weighted average of the trophogenic zone seems the most representative of these alternatives. It is based on more depths and samples, it adjusts for the volumes of the different strata, and it involves the biologically important, productive region of the lake. Unfortunately, this volume-weighted average also requires the most effort in both the field and the laboratory, and we were concerned that this effort may not be well repaid in terms of accuracy and precision of the estimate. Four of the other five measures represent logistic compromises with this ideal of representativity. The composite sample of the water column is included because this protocol included deep peaks, even when they lay below the euphotic depth. Comparisons of the column samples with others will therefore show the effect of inconsistencies associated with the inclusion of chlorophyll peaks in determining the trophic status of lakes. Such an assessment is needed because such peaks occurred in three quarters of our samples (M. Hanna and R. H. Peters, in prep.).

Statistical Analysis

The appropriate sampling method for any study is conditioned by the goal of the analyst. This study presumes that the comparative water quality data are used primarily to characterize individual lakes as unambiguously as possible and so to distinguish among lakes on the basis of mean levels of phosphorus and chlorophyll. Such distinctions are easiest when the property representing each lake under examination is known precisely. We evaluated imprecision with measures of uncertainty such as the variance or standard deviation of the mean.

Phosphorus and chlorophyll concentrations in lake waters are often log-normally distributed or nearly so; hence, log-transformation is used to satisfy the requirements of parametric analysis (Heyman et al. 1984; Knowlton et al. 1984; Marshall et al. 1988). The necessity of transformation was confirmed for the present data: for each protocol, regression revealed strong ($P < 0.001$) positive ($2.5 <$ slope < 3.1) relationships between the logarithms of the arithmetic means and the logarithms of their variances for phosphorus and chlorophyll measured at each site. These relationships all but disappeared when the data were log-transformed before calculating the means and variances. In the four cases where a trend ($P < 0.05$) persisted between the log-means and log-variances (composite samples of phosphorus to the euphotic depth, composite column samples of chlorophyll, and both composite and integrated samples of chlorophyll in the trophogenic zone), only one (the last) was also significant at the 1% level. Thus, log-transformation successfully removed the effect of the mean on imprecision in 8 of 12 cases and weakened that relationship in the remaining 4. In all cases, the slope was small, so that the maximum average change in log-variance over the range of log-means in this data set was only 50%. Although additional transformations (e.g. Taylor 1961) could remove even these trends, such transformations offer only modest parametric advantages, but would complicate statistical comparisons with respect to current practice. We therefore based statistical comparisons in this study on log-transformed variables. Arithmetic means are presented for simplicity, and the associated imprecisions are given as the coefficients of variation (CV = 100 SD/mean) even though slight ($0.8 <$ slope $< 2\%$ mg P or Chl/m^3), but significant ($P < 0.05$) trends for CV to increase with the mean occurred in both subsurface and epilimnetic integrated samples of phosphorus and in all but subsurface and epilimnetic integrated samples of chlorophyll. Statistical analyses used SAS (1985).

Results and Discussion

The ranges of the mean values of phosphorus and chlorophyll associated with each sampling strategy for each lake (Table 2)

TABLE 2. Arithmetic mean concentrations (mg/m^3) of phosphorus and chlorophyll plus associated coefficients of variation (%) (below) for each combination of method (see Fig. 1) and lake basin for the course of the study (midsummer 1986 and 1987, n = 10–12).

	Phosphorus						Chlorophyll					
	Composite			Integrated tube			Composite			Integrated tube		
Lake	1%	2SD	Col.	EPI	2SD	Sub-surf.	1%	2SD	Col.	EPI	2SD	Sub-surf.
Magog	59.1	44.5	68.7	58.9	48.2	41.6	17.5	18.2	14	17.2	21.4	17.2
	96	26	46	92	27	32	45	47	43	43	38	56
Echo	30.2	30.3	30.9	13.9	33.6	20.8	3.7	3.8	3.9	2.6	12.7	2.4
	65	64	61	8.5	68	74	34	33	29	25	51	22
Hertel	26.3	27.2	28.9	20.8	30.5	38.1	14.6	16.0	14.4	5.9	34.1	4.8
	57	51	24	22	35	122	64	80	69	26	90	28
Memphremagog (South)	19.3	23.8	24.4	17.8	21.3	19.7	3.9	4.0	3.9	3.3	3.9	3.0
	37	35	37	35	30	35	24	22	19	21	19	20
Memphremagog (Central)	16.1	15.6	16.0	14.7	16.5	12.6	2.8	2.7	2.4	2.6	2.5	2.0
	34	33	5	30	29	35	28	26	25	28	30	41
Massawippi	14.3	14.5	18.6	15.2	13.2	11.7	4.3	4.4	3.9	4.3	4.0	4.0
	7.9	8.4	86	17	37	15	24	23	35	24	20	20
Lovering	13.1	12.6	13.4	11.4	13.1	13.7	2.7	2.6	2.4	2.6	2.7	2.6
	24	19	38	32	13	37	31	23	21	27	26	34
Memphremagog (North)	12.3	12.3	11.9	13	12.6	11.1	2.5	2.5	2.3	1.8	2.4	2.5
	31	31	28	20	33	32	27	28	27	44	36	40
Petit Brompton	11.6	11.9	10.9	11.5	15.2	9.8	1.9	1.8	1.7	1.7	1.9	1.2
	44	41	21	55	54	55	44	44	40	39	45	44
Croche	11.2	11.8	12.3	7.9	10.8	11.3	3.6	3.8	4.2	2.0	5.7	1.9
	46	42	37	28	29	103	17	18	23	14	47	16
Montjoie	11.0	11.2	11.2	9.7	11.1	10.6	2.2	2.2	2.1	2.4	2.2	2.2
	29	27	26	13	32	36	25	25	21	26	29	41
Brompton	9.8	10.2	10.1	10.7	9.4	8.9	2.3	2.4	1.8	2.1	2.2	2.0
	19	27	23	23	18	26	44	61	32	36	29	42
Achigan	9.2	9.2	9.2	10.4	10.0	7.7	3.6	3.6	2.7	3.1	3.7	3.0
	20	18	16	33	27	27	43	44	34	52	43	61
Orford	9.2	9.2	9.1	10.0	8.1	11.8	1.4	1.5	1.3	1.2	1.4	1.2
	36	39	34	25	14	143	22	20	21	24	25	29
Stukley	8.2	8.2	8.1	9.1	7.5	8.4	2.6	2.5	2.2	2.2	2.6	2.3
	28	28	33	40	20	86	44	44	40	39	45	44
Bowker	8.0	6.4	8.0	5.8	7.0	5.5	1.4	1.4	1.4	1.1	1.3	1.0
	68	28	68	24	45	25	19	17	19	18	16	52
Average	40	32	39	31	32	55	33	35	31	30	37	37
SD of CV	22	13	19	20	14	39	13	18	13	10	18	14

show that the lakes vary from oligotrophic to moderately eutrophic. The CV's associated with each variable and protocol are substantial but no combination obviously differs in the achieved precision (Table 2). The various measures are highly correlated among themselves (Table 3). Even though average concentrations (Table 2) differ enough that the choice of sampling protocol would affect the ranking of some lakes with regard to trophic status, any protocol would succeed in establishing qualitative trends among the lakes.

Imprecision

Pairwise t-tests failed to identify any significant difference among the log-variances generated by different sampling protocols for each lake ($P > 0.05$). Thus, given the level of sampling effort (n = 16), we were unable to identify any protocol as more likely to yield imprecise results than any other. If the CV is instead used as an index of uncertainty, no sampling strategy consistently minimized or maximized that measure of the error about the mean in all lakes and the average CV's for different sampling protocols were very similar (Table 2). Significant differences among methods were found for both phosphorus ($P < 0.05$) and chlorophyll ($P < 0.01$) when the CV's associated with each method for each lake were ranked and Friedman's nonparametric rank test applied to the whole data set. Although this test does not identify which method is significantly different, summed ranks showed that the subsurface samples were the most imprecise because they most frequently had the highest CV for both phosphorus and chlorophyll. The summed ranks for other protocols were more similar. Thus, it

TABLE 3. Simple correlation coefficients (r, %) between the arithmetic mean concentrations of phosphorus (above the diagonal) and chlorophyll (below the diagonal) in 16 lake basins, as determined with six different sampling protocols identified by the abbreviations at the head of each row and column (see Fig. 1). $P < 0.05$ where $r > 50\%$.

	Composite			Integrated tube		Sub-
	1%	2SD	Col.	EPI	2SD	surf.
Comp. 1%	—	100	98	94	94	92
Comp. 2SD	99	—	99	93	95	92
Comp. Col.	82	81	—	88	96	87
Tube EPI	92	91	85	—	84	98
Tube 2SD	93	93	58	80	—	83
Subsurf.	98	98	82	93	90	—

TABLE 4. Bias in mean estimates of phosphorus (below the diagonal) and chlorophyll concentrations (above the diagonal). Each entry is the geometric mean (%) of the ratio of the concentrations measured in paired estimates at each site and date estimated with two of six different sampling protocols (see Fig. 1) (i.e. entry = $10^{(\text{mean log } (C/R))}$ where C and R are the individual measurements for each day and site collected with the method described at the beginning of each column (C) and row (R)). For example, subsurface chlorophyll concentrations average only 87% of the epilimnetic concentrations sampled with an integrating tube, and phosphorus concentrations estimated with other protocols exceeded subsurface phosphorus levels by 7–22%. Significant levels in pairwise t-tests between the logarithmic values for each protocol have been calculated as $\alpha/5$ to adjust for repeated use of the same data in the five comparisons. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

	Composite			Integrated tube		Sub-
	1%	2SD	Col.	2SD	EPI	surf.
Comp. 1%	—	101 ns	94***	112*	84***	73***
Comp. 2SD	100 ns	—	93***	111**	83***	73***
Comp. Col.	94*	94*	—	120***	90***	78***
Tube 2SD	100 ns	99 ns	105 ns	—	73***	63***
Tube EPI	107 ns	108*	114**	112**	—	87***
Subsurf.	115***	115***	122***	115**	107 ns	—

seems that most methods offer similar levels of precision with realistic levels of sampling effort.

Given that composite samples average several independent measurements, it may seem initially surprising that the composite measures based on volume-weighted averages were no less variable than tube samples based on single samples. The comparison suggests that the averaging associated with the calculation of composite samples resulted in a negligible gain in precision. The errors associated with the means result less from analytical variation in making the measurements than from differences among sites, dates, and years. This might be expected because the CV's in replicate determinations of phosphorus and chlorophyll in this laboratory are less than 5% of the mean, but the uncertainty associated with these lake samples is usually over 30% of the mean. This confirms the assessment of Knowlton et al. (1984) that replication error was small, based on many more lakes.

Bias

Pairwise comparisons of the phosphorus and chlorophyll estimates associated with each protocol at each site and date allow assessment of sampling biases. The t-tests show that volume-weighted measures of the trophogenic and euphotic zones gave similar values, but that all other protocols resulted in significantly different chlorophyll concentrations (Table 4). On average, the subsurface samples contained the least chlorophyll, epilimnetic tube samples somewhat more, the various volume-weighted samples were next highest, and the tube samples of the trophogenic zone contained the most. The average concentration in the latter was less than 50% higher than the subsurface mean (Table 4). The high levels of significance in Table 4 reflect the large number of samples in the comparisons ($140 < n < 164$) rather than the magnitude of the differences or the consistency of the results. Exceptions to all patterns occurred at some dates and sites during the study. Nevertheless, even when comparisons of the protocols were limited to individual basins, the same patterns in mean chlorophyll levels were observed and these were often significant.

Phosphorus concentrations were more consistent across methods and therefore across depths than those for chlorophyll: integrated tube samples of the trophogenic zone did not differ significantly from any of the volume-weighted values, and epilimnetic and subsurface phosphorus concentrations were similar (Table 4). Differences among the paired samples indicated that subsurface samples contained the least phosphorus, epilimnetic samples somewhat more, samples of the trophogenic or euphotic zones still more, and columnar composites the most; but the maximum difference in the means was only slightly more than 20%. The vertical structure of phosphorus in these lakes is less marked than that of chlorophyll, so phosphorus concentrations should be less affected by sampling protocol. As with chlorophyll, occasional dates and sites provided exceptions to the trend to higher phosphorus concentrations, so one cannot assume, for example, that subsurface samples will always contain less phosphorus than deeper samples.

Sources of Variation

Nested analysis of variance conducted on each sampling protocol (Table 5) partitioned over 90% of the variances in phosphorus and over 95% of those in chlorophyll to differences among lakes, dates, years, and replication. With one exception (subsurface phosphorus), only differences among lakes, sampling dates, and years had significant effects, and in all cases, most of the variation in phosphorus and chlorophyll determinations was due to differences among lakes.

The very highly significant effect of interlake differences ($P < 0.0001$) explained from 58 to 82% of the variation in phosphorus or chlorophyll concentrations in each method. This result can be interpreted as showing that any of the protocols can distinguish among lakes. In all cases, the variance of chlorophyll was more closely associated with differences among lakes than was that of phosphorus, but the differences between chlorophyll and phosphorus are likely too small to be of real concern in lake monitoring.

Date, reflecting variation among visits to the same lake, also contributed significantly to the observed variation in estimates of chlorophyll ($P < 0.0001$) and phosphorus ($0.05 < P < 0.001$). The effect of date was particularly strong in subsurface samples, for the partial R^2 values show that date explains 26% of the variation in subsurface concentrations of phosphorus and chlorophyll. This argues against the use of subsurface samples in comparing lakes because such data may be more strongly influenced by seasonal changes. The disadvantages of

TABLE 5. Total explained variance (R^2, %) and the partial effects of differences among lakes, dates, years, and sites on the variance in estimates of phosphorus and chlorophyll concentrations sampled with six different protocols (see Fig. 1). In each analysis of variance, the effects of site and date were nested within lake to avoid any assumption about the similarity of sites or dates in different lakes (SAS 1985) and the significance levels are those identified by the nested analysis of variance. The proportion of the total R^2 explained by each effect was assumed to be proportional to its associated sum of squares. For tube samples taken to twice the Secchi disc depth (Tube 2SD), $n = 141$; otherwise, $n = 161$–163. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; ns, not significant.

Protocol	R^2	Lake	Date	Year	Site
Phosphorus					
Comp. 1%	92	69****	21**	1.1**	1.6 ns
Comp. 2SD	94	74****	17***	0.7*	1.3 ns
Comp. Col.	93	75****	15**	0.5*	2.1 ns
Tube EPI	92	75****	16*	0.03 ns	1.4 ns
Tube 2SD	94	74****	18***	0.4*	1.1 ns
Subsurf.	90	58****	26***	0.5 ns	5.3*
Chlorophyll					
Comp. 1%	97	80****	15****	1.7****	0.1 ns
Comp. 2SD	96	80****	15****	1.3****	0.6 ns
Comp. Col.	97	82****	12****	0.7***	1.0 ns
Tube EPI	97	82****	14****	0.8****	0.8 ns
Tube 2SD	96	82****	13****	0.2 ns	0.9 ns
Subsurf.	95	68****	26****	0.0 ns	0.7 ns

somewhat more seasonal variation in phosphorus than in chlorophyll also tend to augment the advantages in discrimination offered by the slightly higher proportion of the variation in chlorophyll associated with differences among lakes.

Since the data were drawn from only two years, they underestimate the range of interannual variation, and the small, but often highly significant, effect of year in this analysis may not be representative. This probably indicates that we followed the lakes for two similar summers and could not properly test the hypothesis (Knowlton et al. 1984) that interannual variation is substantial.

The lack of an effect of site in 11 of 12 analyses (6 protocols × 2 variables) and its marginal effect ($P < 0.05$) in the single case (subsurface estimates of P concentration) where it proved significant suggest that there is little need to sample different sites during a single visit to a lake. This reinforces conclusions based on the comparison of spatial and temporal variations in single basins in both marine (Platt and Filion 1973; Therriault and Platt 1978) and freshwater (Harjula 1979) systems. Sampling effort should instead be directed to maximizing the number of visits (cf. Marshall et al. 1988).

George (1981) also found that interstation variability in total phosphorus was small in Lake Windermere and attributed the observed difference to the effect of a sewage outfall on one site. Downwind accumulation of seston (Hilton et al. 1989) may influence some measures, but this would not seem likely to produce consistent differences between sites and so would not be revealed in our samples, except perhaps through increased variability in surface or epilimnetic samples which are presumably most affected by wind.

Phosphorus Response Models

To determine if differences in sampling method contribute to the variety of existing phosphorus response models (Nicholls and Dillon 1978) or to the uncertainty of individual models (Benndorf 1988), we regressed mean chlorophyll against mean phosphorus concentration for each sampling method in our data set. Despite a few eccentric data points and resultant differences in the individual parameter estimates, all protocols yield comparable net results. Regression lines from one protocol are similar to one another and to the overall regression based on the pooled data (Fig. 2). As a result, chlorophyll concentrations can be effectively predicted by combining phosphorus concentrations measured with one protocol and an equation based on another protocol. For example, the most disparate relationship in Fig. 2 is the one for the column composite; regressions of predicted chlorophyll concentrations, based on equations from one protocol and phosphorus concentrations measured with another, against observed chlorophyll levels did not differ significantly from the ideal (intercept = 0; slope = 1).

Individual researchers can produce empirical relationships that yield similar predictions despite differences in their sampling routines. When these data are combined in a single regression, the mean trend and uncertainty are again similar to those for each method. Thus, this analysis shows that the scatter associated with such regressions is unlikely to reflect the combination of different sampling protocols when different studies are pooled. Given that site has a negligible effect on measurements (Table 5), the conclusion can be confidently extended to analyses that compare separate samples collected simultaneously with the same protocol, and so most monitoring programs are likely to yield comparable results. This comparability of different estimates is limited to phosphorus and chlorophyll samples collected on the same dates or with the same protocols because differences in date and protocol between the two variables significantly bias mean estimates and so may affect the regressions built on those means.

Conclusions

Our comparisons show that both chlorophyll and phosphorus concentrations are robust indices of lake trophic status with respect to variations in sampling methodology. No doubt this robustness has been important in the development of phosphorus response models from literature data based on diverse methodologies. Because our conclusions are only empirically justified, they cannot be presumed to apply beyond the domain described by the characteristics of these lakes. In particular, their application to highly eutrophic waters remains to be established. Because subsurface samples yield low values and are subject to greater daily variation, they should be avoided in cross-sectional lake surveys. Despite their advantages in principle, volume-weighted samples offer little measurable advantage over integrated tube samples, and because the latter involve less effort, they appear to be the method of choice. Integrated tube samples collecting to the bottom of the epilimnion or the trophogenic zone, estimated as twice the Secchi disc depth, proved similarly effective in describing differences among lakes and dates. A choice between these two protocols favours tube sampling the trophogenic zone because such samples represent lake productivity better in principle, even if this advantage could not be demonstrated in practice with the data presented here. Because most of the within-lake variance associated with the mean reflects temporal variation over the season in all protocols, sampling effort should be directed to sampling the same lake frequently, rather than increasing replication levels at each sampling date (Marshall et al. 1988).

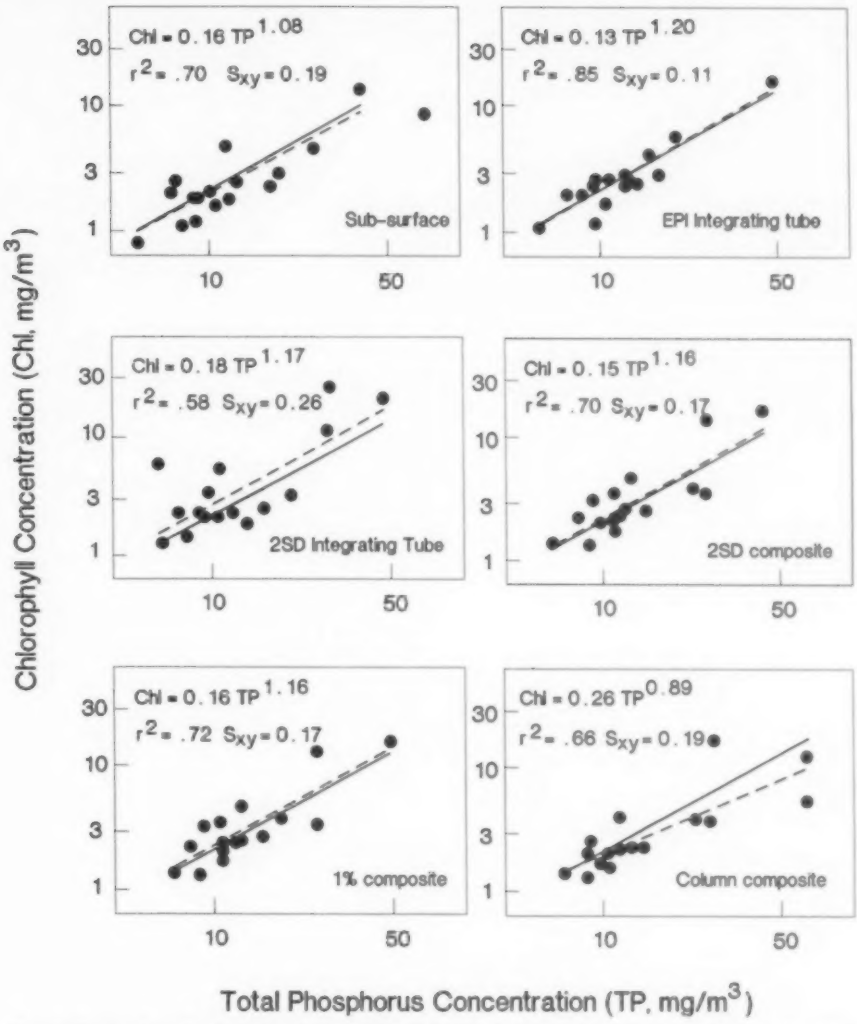

FIG. 2. Regressions (broken lines) between mean chlorophyll and phosphorus concentrations based on each sampling protocol (Table 2; $n = 16$ in all cases) and on all results combined ($n = 96$). The equation from the pooled regression (Chl = 0.16 $TP^{1.14}$, $r^2 = 0.67$, $S_{xy} = 0.19$) appears as the solid line in each panel.

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References

BENNDORF, J. 1988. Objectives and unsolved problems in ecotechnology and biomanipulation: a preface. Limnologica 19: 5–8.

BERGMANN, M., AND R. H. PETERS. 1980. A simple reflectance method for the measurement of particulate pigment in lake water and its application to phosphorus–chlorophyll–seston relationships. Can. J. Fish. Aquat. Sci. 39: 785–790.

CATTANEO, A. 1987. Periphyton in lakes of different trophy. Can. J. Fish. Aquat. Sci. 46: 296–303.

DILLON, P. J., AND F. H. RIGLER. 1974. The phosphorus–chlorophyll relationship in lakes. Limnol. Oceanogr. 19: 767–773.

GEORGE, D. G. 1981. The spatial distribution of nutrients in the South Basin of Windermere. Freshwater Biol. 11: 405–424.

HANNA, M. 1990. Evaluation of models predicting mixing depth. Can. J. Fish. Aquat. Sci. 47: 940–947.

HARJULA, H. 1979. Analysis of errors in estimating phytoplankton primary productivity and chlorophyll *a* with special reference to Lake Paijanne. Ann. Bot. Fenn. 16: 307–337.

HEYMAN, U., S. O. RYDING, AND C. FORSBERG. 1984. Frequency distributions of water quality variables: relationships between mean and maximum values. Water Res. 18: 787–794.

HILTON, J., T. CARRICK, E. RIGG, AND J. P. LISHMAN. 1989. Sampling strategies for water quality monitoring in lakes: the effect of sampling method. Environ. Pollut. 57: 223–234.

KNOWLTON, M. F., M. V. HOYER, AND J. R. JONES. 1984. Sources of variability in phosphorus and chlorophyll and their effects on use of lake survey data. Water Resour. Res. 20: 397–407.

MARSHALL, T. M., A. MORIN, AND R. H. PETERS. 1988. Precision and accuracy in estimates of mean chlorophyll-*a* concentration: implications for sampling design. Water Resour. Res. 24: 1027–1034.

MARSHALL, C. T., AND R. H. PETERS. 1989. General patterns in the seasonal development of chlorophyll-*a* for temperate lakes. Limnol. Oceanogr. 34: 856–867.

MENZEL, D. W., AND N. CORWIN. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulphate oxidation. Limnol. Oceanogr. 10: 280–282.

NICHOLLS, K. N., AND P. J. DILLON. 1978. An evaluation of phosphorus–chlorophyll relationships for lakes. Int. Rev. Gesamten Hydrobiol. 22: 423–444.

OECD. 1982. Eutrophication of waters: monitoring, assessment and control, OECD, Paris. 154 p.

PETERS, R. H. 1986. The role of prediction in limnology. Limnol. Oceanogr. 31: 1143–1159.

1989. Some pathologies in limnology. Mem. Ist. Ital. Idrobiol. 47: 175–212.

PETERS, R. H., AND A. CATTANEO. 1984. The effects of turbulence on phosphorus supply in a shallow bay of Lake Memphremagog. Verh. Int. Ver. Limnol. 22: 185–189.

PLATT, T., AND C. FILION. 1973. Spatial variability of the productivity:biomass ratio for phytoplankton in a small marine basin. Limnol. Oceanogr. 18: 743–749.

SAS. 1985. User's guide: statistics, version 5 edition. SAS Institute Inc., Cary, NC. 956 p.

SEIP, K. L., AND H. IBREKK. 1988. Regression equations for lake management. Verh. Int. Ver. Limnol. 23: 778–785.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. 2nd ed. Bull. Fish. Res. Board Can. 167.

TAYLOR, L. R. 1961. Aggregation, variance and the mean. Nature (London) 189: 732–735.

THERRIAULT, J.-C., AND T. PLATT. 1978. Spatial heterogeneity of phytoplankton biomass and related factors in the near-surface waters of an exposed coastal embayment. Limnol. Oceanogr. 23: 888–899.

THORNTON, K. W., R. H. KENNEDY, A. D. MAGOUN, AND G. E. SAUL. 1982. Reservoir water quality sampling design. Water Resour. Bull. 18: 471–480.

WELCH, P. S. 1948. Limnological methods. McGraw-Hill Book Co. Inc., New York, NY. 381 p.

Lake Acidification and Fisheries Project: Acclimation to Low pH and Elevated Aluminum by Trouts

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The following five papers represent the third set published in the *Canadian Journal of Fisheries and Aquatic Sciences* (CJFAS) from the Electric Power Research Institute's (EPRI) Lake Acidification and Fisheries (LAF) Project. The first set of nine papers dealt with responses of adult brook trout (*Salvelinus fontinalis*) exposed in the laboratory to water quality conditions typical of acidified lakes and streams, with lowered pH, elevated aluminum concentrations, and low calcium concentrations (Can. J. Fish. Aquat. Sci. 45(9)). The seven papers in the second set presented toxicology, physiology, and histopathology results for brook trout eggs, fry, and juveniles (Can. J. Fish Aquat. Sci. 47(8)).

The overall goal of the LAF Project has been to determine cause–effect relationships between surface water acidification and fish population responses by integrating bioassay data from the laboratory and water quality and fish population data from the field into a predictive modeling framework. An overview of specific steps in the project and a summary of major findings are given in Bergman et al. (1988) for adult brook trout and Bergman and Mattice (1990) for brook trout early life stages. Additional details on the results for brook trout and rainbow trout (*Oncorhynchus mykiss*) are summarized in Mount and Marcus (1989) and Marcus and Mount (1991).

In the set of five papers presented in this issue, LAF investigators report results from a series of experiments on acclimation to low pH and elevated aluminum by brook trout and rainbow trout. In these papers, both physiological (McDonald et al. 1991; Walker et al. 1991; Reid et al. 1991) and histological (Mueller et al. 1991; Freda et al. 1991) mechanisms of toxicity and acclimation are presented and discussed.

Additional publications anticipated from the LAF Project include toxicology, physiology, and histopathology results of further experiments with brook trout, rainbow trout, white sucker (*Catostomus commersoni*), and smallmouth bass (*Micropterus dolomieui*), demonstrations and applications of the models and modeling framework built upon these results, and comparisons and syntheses covering results from all four fish species studied in the LAF Project.

Data Availability

Because of the extensive nature of the toxicology data sets produced in this project (e.g. daily observations on as many as 84 different exposure combinations of pH, aluminum, and calcium in 17 different experiments that together cover six life stages of brook trout), only summaries and applications of data are presented in the publications in past, current, and anticipated issues of CJFAS. Because many investigators and agencies may find additional uses for the full toxicology data sets, they will be made available on computer disc or tape from the Electric Power Software Center, 1930 Hiline Drive, Dallas, TX 75207, USA.

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References

Bergman, H. L., and J. S. Mattice. 1990. Lake acidification and fisheries project: brook trout (*Salvelinus fontinalis*) early life stages. Can. J. Fish. Aquat. Sci. 47: 1578–1579.

Bergman, H. L., J. S. Mattice, and D. J. A. Brown. 1988. Lake Acidification and Fisheries Project: adult brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1561–1562.

Freda, J., D. A. Sanchez, and H. L. Bergman. 1991. Shortening of branchial tight junctions in acid-exposed rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 2028–2033.

Marcus, M. D., and D. R. Mount [ed.] 1991. Physiological, toxicological, and population responses of rainbow trout to acidification. EN-7114. Electric Power Research Institute, Palo Alto, CA 94304, USA.

McDonald, D. G., C. M. Wood, R. G. Rhem, M. E. Mueller, D. R. Mount, and H. L. Bergman. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). I. Physiology. Can. J. Fish. Aquat. Sci. 48: 2006–2015.

MOUNT, D. R., AND M. D. MARCUS [ED.] 1989. Physiological, toxicological, and population responses of brook trout to acidification. EN-6238. Electric Power Research Institute, Palo Alto, CA 94304, USA.

MUELLER, M. E., D. A. SANCHEZ, H. L. BERGMAN, D. G. MCDONALD, R. G. RHEM, AND C. M. WOOD. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. Can. J. Fish. Aquat. Sci. 48: 2016–2027.

REID, S. D., D. G. MCDONALD, AND R. R. RHEM. 1991. Acclimation to sublethal aluminum: modifications of metal – gill surface interactions of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1996–2005.

WALKER, R. L., C. M. WOOD, AND H. L. BERGMAN. 1991. Effects of long-term preexposure to sublethal concentrations of acid and aluminum on the ventilatory response to aluminum challenge in brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 48: 1989–1995.

Effects of Long-Term Preexposure to Sublethal Concentrations of Acid and Aluminum on the Ventilatory Response to Aluminum Challenge in Brook Trout (*Salvelinus fontinalis*)

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Adult brook trout (*Salvelinus fontinalis*; 200–300 g) were preexposed for 10 wk to 75 μg $Al^{3+} \cdot L^{-1}$, pH = 5.2, in soft water (25 μequiv $Ca^{2+} \cdot L^{-1}$) and controls to the same conditions without Al^{3+}. When challenged with a lethal dose of Al^{3+} (333 $\mu g \cdot L^{-1}$) at the same pH (5.2) and Ca^{2+} (25 $\mu equiv \cdot L^{-1}$), the control fish demonstrated a twofold increase in ventilation volume and ventilatory stroke volume within the first two hours, an increase in $Paco_2$, and a decrease in pHa and hemoglobin O_2 saturation. These effects were not seen in the group chronically preexposed to sublethal Al, indicating that some acclimation had occurred. Although the prechallenge Pao_2 and $Paco_2$ were the same in the two groups, the arterial pH, plasma $[HCO_3^-]$, and hemoglobin O_2 saturation of the Al-preexposed fish were significantly below those of the control fish, suggesting that the acclimation was achieved at some cost. Possible mechanisms are discussed.

Pendant 10 sem, on a exposé des ombles de fontaine *Salvelinus fontinalis* adultes pesant de 200 à 300 g à 75 μg $Al^{+3} \cdot L^{-1}$ en eau douce de pH 5,2 (25 μequiv $Ca^{2+} \cdot L^{-1}$). Les ombles témoins ont été exposés aux mêmes conditions en l'absence d'Al^{+3}. Lorsque mis en présence d'une dose létale d'Al^{+3} (333 $\mu g \cdot L^{-1}$) en milieu de même pH (5,2) et concentration de Ca^{+2} (25 $\mu equiv \cdot L^{-1}$), ces derniers ont montré une augmentation par un facteur de deux du volume de ventilation et du volume de l'amplitude de pulsation ventilatoire en deçà des deux premières heures d'exposition, une augmentation de la teneur en $Paco_2$ ainsi qu'une baisse du pHa et du niveau de saturation en O_2 de l'hémoglobine. Ces incidences n'ont pas été relevées dans le groupe pré-exposé de façon chronique à une teneur sublétale d'Al, ce qui indique qu'une certaine acclimatation a eu lieu. Quoique les teneurs en Pao_2 et $Paco_2$ avant l'exposition étaient les mêmes dans les deux groupes, le pH artériel, la concentration de HCO_3^- du plasma et la saturation en O_2 de l'hémoglobine chez tous les poissons pré-exposés à l'Al étaient nettement inférieurs aux valeurs relevées chez les poissons témoins. Ceci laisse supposer que l'acclimatation s'est effectuée aux dépens de certains processus. Les auteurs traitent de mécanismes pouvant expliquer ce phénomène.

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The combined effect of acid and aluminum on ventilation has been documented in brown trout (*Salmo trutta*) (Muniz and Leivestad 1980; Rosseland 1980), rainbow trout (*Oncorhynchus mykiss*) (Neville 1985; Malte and Weber 1988; Playle and Wood 1989), and brook trout (*Salvelinus fontinalis*) (Rosseland 1980; Walker et al. 1988). Although a variety of combinations of water pH, Al, Ca, and temperature was tested among these studies, the general conclusion is that exposure of fish naive to acid pH and Al in lethal combinations results in hyperventilation, mucus clogging of the gills, and eventual death which is caused at least in part by respiratory impairment. Typical internal symptoms of this respiratory distress are a drop in arterial O_2 tension, a reduction in hemoglobin O_2 content, a rise in arterial CO_2 tension, an accumulation of lactate, and a decrease in arterial pH as a result of the combined respiratory and metabolic acidosis (Neville 1985; Malte and Weber 1988; Wood et al. 1988b; Walker et al. 1988; Playle et al. 1989).

However, it now appears that prior exposure to sublethal concentrations of acid/Al can alleviate the respiratory distress and reduce the mortality associated with exposure to higher concentrations of these toxicants. Orr et al. (1986) found that prior exposure of rainbow trout fingerlings to sublethal concentrations of Al (87–154 $\mu g \cdot L^{-1}$) at pH = 5.2 for 2–3 wk significantly increased the LC50 of Al at the same pH. Wood et al. (1988a, 1988c) found that adult brook trout preexposed

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to Al = 150 $\mu g \cdot L^{-1}$ for 10 wk at pH = 5.2 were able to maintain electrolyte balance and blood respiratory parameters when challenged with Al = 333 $\mu g \cdot L^{-1}$ at pH = 4.8, a combination which proved lethal to fish lacking previous Al exposure (i.e. Al naive). In fact, there were no mortalities among the Al-preexposed group over the 72-h challenge period, while the Al-naive fish all died (Wood et al. 1988c). The evidence to date strongly suggests that acclimation occurs to Al alone, and not to low pH, during long-term exposure to sublethal acid/Al conditions (Wood et al. 1988a, 1988c; Audet and Wood 1988). Although the actual mechanism of acclimation has not been elucidated, Wood et al. (1988a, 1988c) speculated that gill ligand attraction for Al might be reduced during acclimation which in turn would minimize Al deposition on the gills and prevent mucification and inflammation during Al challenge.

Extended periods of exposure to sublethal acid/Al have been reported to cause thickening of the gill epithelium leading to increased water to blood diffusion distance (Chevalier et al. 1985; Tietge et al. 1988; Karlsson-Norrgren et al. 1986a, 1986b). Nevertheless, the observations of Wood et al. (1988a, 1988c) clearly indicated that there were no chronic abnormalities of arterial O_2 or CO_2 tensions or hemoglobin O_2 content as a result of the exposure and negligible disturbances of these parameters during more severe acid/Al challenge. Indeed, the implications are that pulses of acid and Al, seen when the snowpack melts in the spring, may be less severe with regards to fish survival than previously thought, considering the effects of prior exposure to sublethal acid/Al.

Nothing is yet known of the effects of Al acclimation on ventilatory responses. Would the long-term preexposure of adult brook trout to sublethal concentrations of acid and Al alleviate the acute hyperventilatory response (Walker et al. 1988) to a more severe Al challenge? In light of the previous discussion, we anticipated that acute respiratory distress would not occur in the acid/Al-preexposed fish and that mortality would be substantially reduced in response to the challenge.

Materials and Methods

Experimental Animals and Conditions

Experiments were performed at the Fish Physiology and Toxicology Laboratory, Laramie, WY (altitude = 2200 m), which provided facilities for continuous exposure of adult fish to defined pH, Ca^{2+}, and Al conditions in flowing artificial soft water. In brief, the artificial soft water was prepared from hard well water by sedimentation filtration, NaCl softening, reverse osmosis, and separate bed deionization (Continental Water Systems, Denver, CO). The pH was adjusted to 6.5 with KOH, Na^+ (40 $\mu equiv \cdot L^{-1}$) and Ca^{2+} (25 $\mu equiv \cdot L^{-1}$) were added as chlorides, and the water was thoroughly mixed and then delivered to head tanks where the pH was lowered to 5.2 with H_2SO_4. All pH adjustments were made with Leeds and Northrup pH controllers (pH analyzer/controller model No. 7083). The required levels of $AlCl_3$ were added via Mariotte bottles. From there, water was distributed directly to 340-L fiberglass fish holding tanks at a rate of 1.9 $L \cdot min^{-1}$ and then to waste.

Adult brook trout (200–300 g), obtained from Cline's Trout Farm (Boulder, CO), were exposed for 10 wk to either acidified soft water (pH 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$) or acidified soft water containing Al (pH = 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$, Al^{3+} = 75 $\mu g \cdot L^{-1}$) at 11 ± 1°C. Fish were fed 1 floating trout chow (Purina No. 5106) at 1% body weight·d^{-1} and solid wastes were removed by siphon daily. Dissolved O_2 was maintained above 60% air saturation. Photoperiod was adjusted biweekly to follow the natural cycle for Laramie, WY.

Following the 10-wk exposure period, fish from both holding conditions (Al naive, n = 6; Al preexposed, n = 10) were fitted with ventilation masks and arterial catheters (see below). Both groups were then challenged for 72 h with the same acidified soft water (pH = 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$) to which Al^{3+} = 333 $\mu g \cdot L^{-1}$ was added. This concentration of Al in the challenge condition was previously shown to be lethal to brook trout acclimated to pH = 6.5 and pH = 5.2 in the absence of Al (Wood et al. 1988a, 1988c).

Ventilation and Blood Measurements

Prior to the challenge, each fish was equipped with a caudal artery cannula and a latex ventilation mask under anesthesia using the methods outlined in Wood et al. (1988b), Davis and Cameron (1971), and Walker et al. (1988). Fish were anesthetized with MS-222 (ethyl-*m*-aminobenzoate, 50 $mg \cdot L^{-1}$; Sigma Chemical Co., St. Louis, MO) using the soft water from the appropriate acclimation tank. Immediately after surgery, each fish was placed in its own ventilation box which was supplied with the appropriate acclimation water at a flow rate of 0.5 $L \cdot min^{-1}$. Inspired P_{O_2} was maintained above 120 torr (1 torr = 133.322 Pa), P_{CO_2} less than 1 torr, and temperature at 11 ± 1°C. The ventilation boxes were covered in black plastic to reduce disturbance, except for a hole in the top of the box strategically placed to allow observation of opercular movements.

Following a 48-h recovery period, prechallenge ventilation rate (f_R, number per minute) was determined by observation of opercular movements, and ventilation volume ($\dot{V}_w$, millilitres per kilogram per minute) was measured by timed collection of water draining from the expired chamber of the ventilation box. Stroke volume (V_s, millilitres per kilogram per stroke) was calculated from ventilation volume and ventilation rate. O_2 consumption (micromoles per kilogram per minute) was determined by the Fick method, using the difference in P_{O_2} between inspired water and mixed expired water from just behind the opercula, the ventilation volume, and an assumed O_2 solubility of 2.23 $\mu mol \cdot L^{-1} \cdot torr^{-1}$ at 11°C (Dejours 1975).

After measuring the ventilatory parameters while the fish was in the acclimation water, we drew an initial blood sample from the cannula using a heparinized, gas-tight Hamilton syringe. The blood sample (400 µL) was replaced by reinfusing blood from the P_{O_2} electrode (about 100 µL) and nonheparinized saline (Wolf 1963). The blood was analyzed for arterial O_2 tension (Pa_{O_2}), arterial pH (pHa), total O_2 in whole blood (vol% O_2), total CO_2 in true plasma (C_{CO_2}), hematocrit (Hct), hemoglobin concentration ([Hb]), plasma lactate concentration, and plasma Cl^- concentration.

Following the prechallenge sampling period, acid/Al water (pH = 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$, Al^{3+} = 333 $\mu g \cdot L^{-1}$) was piped into the anterior portion of the ventilation chamber. Ventilation was measured 1 h into the challenge and then again immediately prior to blood sampling at 2, 4, 8, 24, 48, and 72 h of exposure, if death did not occur earlier. This sampling regime was chosen because of the acute nature of the respiratory and acid–base response to a similar acid/Al challenge reported by Walker et al. (1988). At each blood sampling time, the suite of measurements outlined above was performed.

Analytical Methods

Water samples were taken from the acclimation tanks for measurement of pH (daily) and Ca^{2+}, Na^+, Cl^-, and Al (weekly). Na^+ and Ca^{2+} were measured on a Perkin-Elmer model 2380 atomic absorption spectrophotometer. Al was measured with an associated high-temperature graphite furnace (Perkin-Elmer model HGA-400). Using the method of LaZerte (1984), Al was speciated into total, monomeric, and inorganic monomeric. Cl^- levels were measured using a Dionex ion-exchange chromatograph (model 2110) with an AS 40 column.

A Radiometer G297/G2 electrode was used for the determination of pHa, and O_2 tension was measured with a Radiometer E5046 P_{O_2} electrode. Both electrodes were maintained at 11°C. Total CO_2 was determined with a Corning 960 CO_2 analyzer using true plasma obtained from sealed heparinized capillary tubes following centrifugation. Hct was read before the plasma was removed. Arterial CO_2 tension (Pa_{CO_2}) and true plasma bicarbonate ($[HCO_3^-]$) were calculated as outlined in Walker et al. (1988).

[Hb] was determined colorimetrically using the cyanmethemoglobin method (Sigma kit No. 525). Mean cell [Hb] (MCHC) was calculated as the [Hb]/Hct ratio (i.e. grams Hb per millilitre of red blood cells).

The O_2 content of whole blood was determined with a Lex-O_2-Con oxygen analyzer (Lexington Instruments Corp.). After conversion of the O_2 content from volume percent to millimoles per litre, the O_2 content per unit Hb (micromoles O_2 per gram of Hb) was calculated by subtracting the dissolved component (Boutilier et al. 1984) and dividing by the measured [Hb].

Plasma Cl^- was measured coulometrically with a Radiometer CMT 10 chloridometer. Lactate determination was performed with a YSI model 27 industrial analyzer (Yellow Springs Instruments).

Treatment of Data

Data have been reported as means ± 1 SEM (n) for both groups at each of the sampling times. For all parameters, the mean ± 1 SEM (n) of the prechallenge and final values are summarized, where the final values represent either the end of the challenge period or the last measurement prior to death. Differences among means were assessed by one-way analysis of variance. Student–Newman–Keuls multiple comparison tests were performed to establish specific differences among means when the F value indicated significance (Zar 1984). In all cases, a criterion of $p \leq 0.05$ was employed for statistical significance. Estimates of median survival times (LT50's) were computed by log-probit analysis of time-percent effect data. Nomographic methods outlined in Litchfield (1949) and Litchfield and Wilcoxon (1949) were used to compute 95% confidence limits for the log-probit regressions. LT50's were considered significantly different when the confidence limits did not overlap.

Results

Exposure Conditions

There was good agreement between the intended and measured water conditions of the acclimation tanks (Table 1). Variation in Na and Cl concentrations was attributed to cyling of the water-softening and deionizing systems. Speciation of Al showed that almost all the Al was available to the fish in the monomeric form, but only about 15–20% of the monomeric was inorganic, probably due to complexation with food or fecal material in the water. Although the speciation of Al was not monitored in the challenge condition, the flow of freshwater over the gill and out the posterior of the ventilation box with each opercular movement precluded the possibility of complexation with organic waste material.

Effects of Al Challenge on Ventilation and O_2 Consumption ($\dot{M}_{O_2}$)

There was approximately a twofold increase in the mean $\dot{V}_w$ of the Al-naive fish within 1 h of the start of the challenge (Fig. 1A). $\dot{V}_w$ continued to increase to about 2.5-fold of the prechallenge $\dot{V}_w$ at 2 h and remained significantly elevated through the first 8 h of the challenge. By 24 h, one third of the Al-naive fish had died, and $\dot{V}_w$ of the survivors was not significantly different from the prechallenge value. Only one of the six Al-naive fish survived to 48 h.

The elevation of $\dot{V}_w$ was largely attributable to a 2- to 2.5-fold increase in $\dot{V}_s$ (Fig. 1B). As with $\dot{V}_w$, $\dot{V}_s$ remained elevated during the first 8 h of the challenge. At 24 h, $\dot{V}_s$ was still significantly greater than that seen during prechallenge. There was also a gradual rise in f_R over the first 8 h of the challenge, although the maximum increase was only 12% (Fig. 1C).

The ventilatory parameters of the Al-preexposed fish were not significantly different from those of the Al-naive fish prior to challenge (Fig. 1; Table 2), and in contrast with the Al-naive fish, there were no significant changes in $\dot{V}_w$, $\dot{V}_s$, or f_R during the challenge period. The survival rate was slightly improved in that 4 of the 10 challenged fish survived to 48 h and 2 were still alive at 72 h. All three ventilation parameters showed a slight increase between 24 and 48 h, but the means were not statistically significant from the prechallenge values.

There was no statistically significant change in $\dot{M}_{O_2}$ in either group throughout the challenge period (Fig. 1D).

Effects of Al Preexposure and Al Challenge on Acid–Base Status and Blood Gases

With regard to the acid–base status of the two groups (Fig. 2), both the prechallenge pHa (7.69 versus 7.93) and true plasma $[HCO_3^-]$ (7.6 versus 11.6 mmol·L^{-1}) were substantially and significantly lower in Al-preexposed trout than in Al-naive fish. Calculated Pa_{CO_2} was also slightly elevated in the Al-preexposed fish compared to the Al-naive group (3.6 versus 3.1 torr). The prechallenge plasma lactate values were virtually identical in the two groups (2.9 versus 3.1 mmol·L^{-1}).

The Al challenge caused a significant reduction in pHa of the Al-naive fish which dropped from 7.93 to 7.80 within the first hour and then to 7.56 at 24 h (Fig. 2A). Although true plasma $[HCO_3^-]$ (Fig. 2B) showed a similar decrease, only the 24-h mean was significantly less than the prechallenge mean (5.4 versus 11.6 mmol·L^{-1}). There was also a significant increase in the Pa_{CO_2} (calculated) at 2 h of the challenge (Fig. 2C), but Pa_{CO_2} returned to prechallenge levels by 4 h and remained there for the balance of the challenge period. The average plasma lactate concentrations over the 24-h period were not significantly different from the prechallenge value (Fig. 2D). However, a comparison of the mean terminal lactate concentration and the prechallenge value (Table 2) revealed that there was a significant increase in plasma lactate (6.2 versus

TABLE 1. Intended exposure conditions and measured water chemistry (means and ranges) during the 10-wk acclimation period.

Intended			Measured						
					Al ($\mu g \cdot L^{-1}$)				
pH	Ca^{2+} ($\mu equiv \cdot L^{-1}$)	Al ($\mu g \cdot L^{-1}$)	pH	Ca^{2+} ($\mu equiv \cdot L^{-1}$)	Total Al	Monomeric	Inorganic monomeric	Na^{+} ($\mu equiv \cdot L^{-1}$)	Cl^{-} ($\mu equiv \cdot L^{-1}$)
5.2	25	0	5.09 (4.7–5.6)	27.1 (22–31)	0.6 (<0.1–2.5)	2.2 (<0.1–8.5)	— —	51.8 (47–60)	51.4 (43–70)
5.2	25	75	5.21 (4.9–5.7)	26.8 (23–32)	66.3 (38–104)	59.3 (47–73)	10.7 (3–20)	47.8 (43–53)	54.1 (49–58)

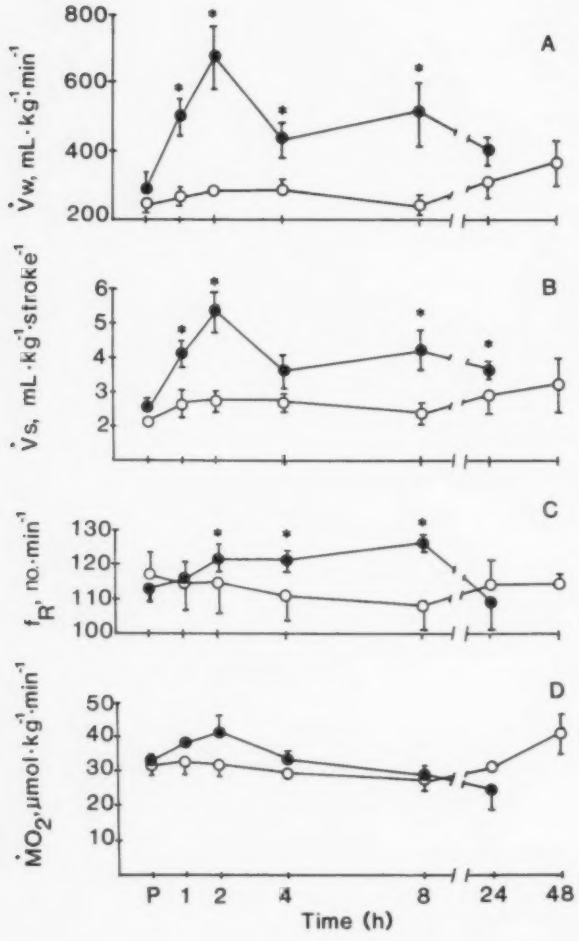

FIG. 1. (A) Ventilation volume ($\dot{V}_w$), (B) ventilatory stroke volume ($\dot{V}_s$), (C) ventilation rate (f_R), and (D) O_2 consumption ($\dot{M}O_2$) of Al-naive (closed circles) and Al-preexposed (open circles) brook trout prior to (P) and during acid/Al challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$). Values are means ± 1 SEM. The number of observations diminished during the experiment due to deaths (n ranged from 6 to 4 for Al-naive and from 10 to 4 for Al-preexposed fish). *Significant difference from the respective prechallenge mean in each group ($p \leq 0.05$).

3.1 $mmol \cdot L^{-1}$) in the Al-naive fish just before death, together with significant decreases in pHa and $[HCO_3^-]$ as noted above.

As with the ventilatory parameters, there were no further significant changes in the acid–base status of the Al-preexposed fish in response to the Al challenge. Plasma pHa, $[HCO_3^-]$, Pa_{CO_2}, and lactate showed only minor fluctuations from the prechallenge values (Fig. 2). However, the mean terminal sample pHa and $[HCO_3^-]$ were significantly less than the prechallenge means, although lactate was not affected (Table 2).

Prechallenge levels of Pa_{O_2} were identical in the two groups, but the prechallenge arterial O_2 content (vol% O_2), the O_2 content per unit Hb (Hb_{O_2}), and MCHC were all significantly higher in the Al-naive fish (Fig. 3A). The difference probably reflected Bohr and Root effects and erythrocyte swelling associated with the lower pHa values for the Al-preexposed trout (Fig. 2A).

Overall there were no significant changes in Pa_{O_2} of either group of fish (Fig. 3A). Average P_{O_2}'s were maintained between 50 and 60 torr (the inspired P_{O_2} was about 120 torr at this altitude). The Al-naive fish experienced a significant drop in Hb_{O_2} at 2 h (Fig. 3C), corresponding with the reduction in arterial pH and the increase in P_{CO_2} at this time (Fig. 2). As with Pa_{CO_2}, the reduction in Hb_{O_2} was only transient, as the mean values at 4 h were not significantly different from the means for the prechallenge period. This transient decrease in Hb_{O_2} was not seen in the Al-preexposed trout (Fig. 3C). Hb, Hct (data not shown), and vol% O_2 (Fig. 3B) declined at 24 h in the Al-naive fish due to the combined effects of red blood cell loss through repetitive blood sampling and acidosis. Despite identical blood sampling in the Al-preexposed group, these fish experienced no significant decline in these parameters during challenge. No significant changes in MCHC were observed in either group in the individual sampling periods during challenge, but the terminal MCHC was significantly reduced relative to the prechallenge value in the Al-preexposed group (Table 2).

Effects of Al Preexposure and Al Challenge on Plasma Chloride and Mortality

Due to a shortage of plasma, the only plasma inorganic ion measured was Cl^- (Table 2). There was no significant difference between the prechallenge means, but the terminal samples from both groups were significantly less than their prechallenge values.

From the log-probit analysis of mortality, LT50's for the Al-naive and Al-preexposed fish were not significantly different at 32 and 36 h, respectively.

Discussion

The acclimation conditions chosen here were similar to those used by Orr et al. (1986), Wood et al. (1988b), and McDonald

TABLE 2. Initial (prechallenge) and terminal values (means ± 1 SEM (n)) during challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$) for various ventilatory and blood parameters of brook trout preexposed to either pH = 5.2, Al = 0 (Al naive) or pH = 5.2, Al = 75 $\mu g \cdot L^{-1}$ (Al preexposed). *Significant difference from the respective prechallenge mean; **significant difference between group means prior to challenge ($p \leq 0.05$).

Parameter	Al naive		Al preexposed	
	Initital	Terminal	Initial	Terminal
pHa	7.929 ± 0.036 (5)	7.665* ± 0.074 (5)	7.687** ± 0.029 (9)	7.494* ± 0.044 (8)
$[HCO_3^-]$ ($mmol \cdot L^{-1}$)	11.6 ± 1.2 (5)	7.0* ± 0.9 (5)	7.6** ± 0.9 (9)	4.8* ± 0.5 (7)
$Paco_2$ (torr)	3.1 ± 0.4 (5)	3.4 ± 0.3 (5)	3.6 ± 0.4 (9)	3.6 ± 0.3 (5)
Pao_2 (torr)	52.1 ± 6.6 (4)	39.3 ± 10.5 (4)	53.3 ± 8.1 (7)	71.5 ± 5.7 (7)
Hbo_2 ($\mu molO_2 \cdot g\ Hb^{-1}$)	63.7 ± 1.4 (4)	45.5 ± 9.9 (4)	54.2** ± 2.5 (6)	50.8 ± 4.0 (6)
MCHC ($g \cdot mL^{-1}$)	0.250 ± 0.005 (5)	0.226 ± 0.028 (5)	0.223** ± 0.012 (8)	0.183* ± 0.006 (7)
$\dot{V}_w$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	287.5 ± 37.5 (6)	418.2 ± 84.2 (6)	250.0 ± 25.7 (10)	300.0 ± 57.0 (9)
$\dot{V}_s$ ($mL \cdot kg^{-1} \cdot stroke^{-1}$)	2.55 ± 0.36 (6)	3.62 ± 0.59 (6)	2.19 ± 0.24 (10)	2.76 ± 0.55 (8)
f_R ($no. \cdot min^{-1}$)	113 ± 3 (6)	114 ± 7 (6)	117 ± 7 (10)	116 ± 3 (8)
$\dot{M}o_2$ ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	32.7 ± 3.2 (6)	27.6 ± 5.4 (6)	32.1 ± 2.6 (10)	24.3 ± 3.2 (9)
Lactate ($mmol \cdot L^{-1}$)	3.1 ± 0.52 (5)	6.2* ± 0.85 (5)	2.9 ± 0.36 (9)	4.4 ± 0.85 (9)
Cl^- ($mmol \cdot L^{-1}$)	114 ± 4.7 (5)	99.5* ± 4.6 (5)	110.9 ± 2.9 (10)	94.5* ± 3.0 (10)

et al. (1991), who clearly demonstrated the ameliorative effects of prior exposure to sublethal concentrations of Al on the response to lethal Al challenge. Al concentrations used during the challenge period closely matched the field conditions during acid pulses following melt of the snowpack (Schofield 1977; Harvey and McArdle 1986). The time span of the study was limited to 72 h, which was considered sufficient to observe any lethal effects of the Al challenge reported by Harvey and McArdle (1986) and Wood et al. (1988a, 1988c).

The acute hyperventilation (Fig. 1A) during challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$) exhibited by Al-naive fish (preexposed to pH = 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$) was virtually identical to the response to a slightly different acid/Al challenge (pH = 4.8, Al = 333 $\mu g \cdot L^{-1}$) of adult brook trout naive to both acid and Al (preexposed to pH = 6.5, Ca^{2+} = 400 $\mu equiv \cdot L^{-1}$) reported in our previous study (Walker et al. 1988). In that investigation, interpretation of the hyperventilatory response was complicated by changes in water pH (and transiently Pco_2) as well as water Al during the challenge. In the present study, this was not a problem because water pH was maintained unchanged at 5.2, and there was no disturbance of inspired Pco_2. The hyperventilatory response was clearly caused by Al alone. Similar increases in ventilation in response to combined acid plus Al challenge in soft water have been reported in other salmonids (Rosseland 1980; Neville 1985; Malte and Weber 1988).

The fact that the decrease in the mean arterial Hbo_2 and increase in $Paco_2$ were only transient and that the mean values for Pao_2 remained unchanged in the Al-naive fish suggests that impairment of respiratory gas exchange was relatively minor in the present study. The relative stability of the blood O_2 and CO_2 tensions may be the result of the hyperventilation. In general, the blood gas responses were similar to those of our previous study (Walker et al. 1988).

It is likely that the ventilatory response was a direct result of the action of Al on the gill. It is known that Al acts as an irritant, causing an increase in gill mucus secretion as reported by Muniz and Leivestad (1980), Mallant (1985), and Walker et al. (1988). In addition to a general irritation response, clogging of the interlamellar spaces with Al/mucus precipitate may have occurred, although gill mucus and Al content were not analyzed.

The ventilatory parameters of the Al-preexposed fish (pH = 5.2, Al = 75 $\mu g \cdot L^{-1}$) remained unchanged (Fig. 1) despite the Al challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$). It is also important to note that these parameters were not significantly different from the prechallenge mean ventilation volume and rate for Al-naive fish. Thus, the work reported here is the first evidence that the ventilatory component of the toxic response to Al can be eliminated through long-term preexposure. How does one explain the complete lack of ventilatory response when the Al-preexposed fish were challenged with lethal levels of Al? Both groups experienced little disturbance in blood gases; therefore, the lack of one in the Al-preexposed fish is probably not the explanation. From previous work of Karlsson-Norrgren et al. (1986a, 1986b), Tietge et al. (1988), and Mueller et al. (1991), one might expect that long-term preexposure would result in a proliferation in mucus cells on the branchial epithelium. However, this does not necessarily imply increased mucus accumulation on the gills during challenge in the Al-preexposed fish. Indeed the reverse may well be true. McDonald et al. (1991) noted that the gill content of sialic acid, an important component of mucus, decreased during the initial stages of acclimation to sublethal Al in juvenile brook trout. This decrease coincided with a removal of Al from the gills. Gill sialic acid content decreased again during more severe Al challenge. McDonald et al. (1991) speculated that an increased turnover and sloughing rate of branchial mucus was an important part of the defence mechanism seen in Al-acclimated fish, for it would minimize the net accumulation of Al on the gills. In juvenile rainbow trout preexposed to sublethal Al, Reid et al. (1991) identified another mechanism which would reduce branchial Al accumulation during challenge, specifically a reduction in the cation binding affinity of the gill surface for Al and in the ability of Al to inhibit gill Ca^{2+} binding.

We therefore speculate that functional and/or compensational changes in the gill surface occurring as a result of preexposure minimized the net accumulation of Al on the gills during challenge. This minimized gill irritation, perhaps in combination with a desensitization of the inflammatory response as a

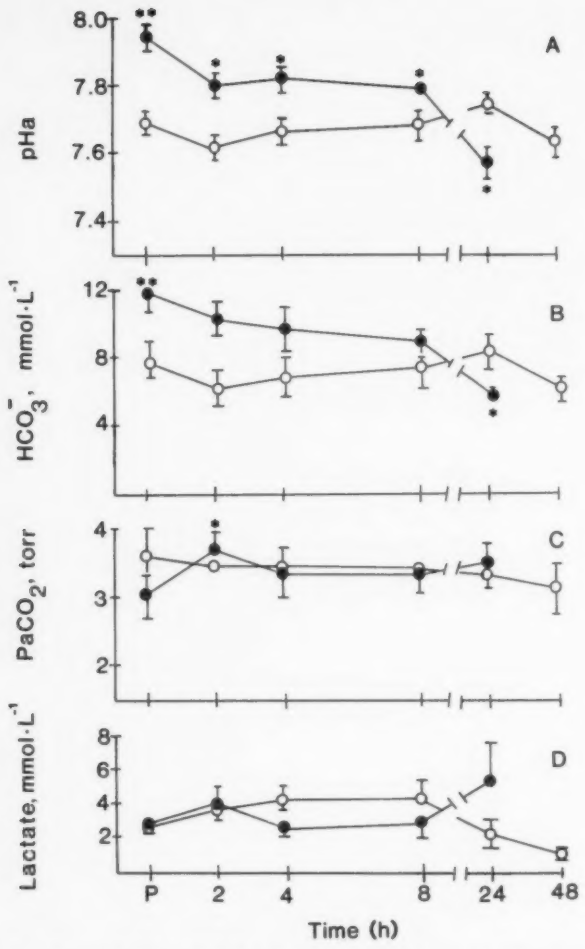

FIG. 2. (A) Arterial pH (pHa), (B) true plasma [HCO_3^-], (C) calculated arterial P_{CO_2} (Pa_{CO_2}), and (D) plasma [lactate] of Al-naive (closed circles) and Al-preexposed (open circles) brook trout prior to (P) and during acid/Al challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$). Values are means ± 1 SEM. The number of observations diminished during the experiment due to deaths (n ranged from 5 to 3 for Al-naive and from 9 to 3 for Al-preexposed fish). *Significant difference from the respective prechallenge mean; **significant difference between group means prior to challenge ($p \leq 0.05$).

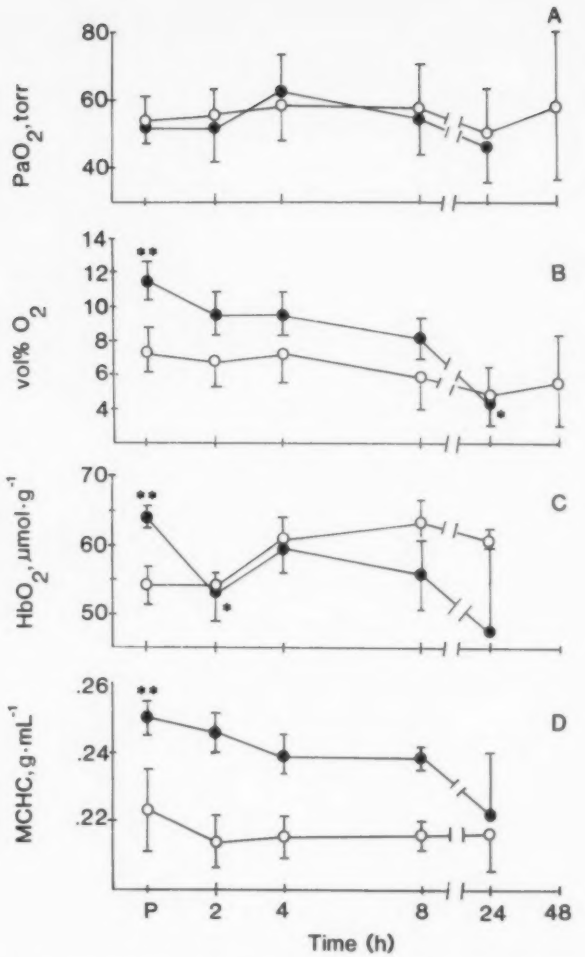

FIG. 3. (A) Arterial P_{O_2} (Pa_{O_2}), (B) vol% O_2, (C) hemoglobin O_2 content (Hb_{O_2}), and (D) mean cell hemoglobin concentration (MCHC) of Al-naive (closed circles) and Al-preexposed (open circles) brook trout prior to (P) and during acid/Al challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$). Values are means ± 1 SEM. The number of observations diminished during the experiment due to deaths (n ranged from 5 to 3 for Al-naive and from 8 to 3 for Al-preexposed fish). *Significant difference from the respective prechallenge mean; **significant difference between group means prior to challenge ($p \leq 0.05$).

result of chronic exposure. The reduced irritation would explain the absence of a hyperventilatory response.

Comparing the prechallenge blood parameters of the Al-naive and preexposed fish, it is apparent that preexposure to Al had no significant effect on either Pa_{O_2} (Fig. 3B) or Pa_{CO_2} (Fig. 2C), but altered the resting acid–base status (decreased pHa, [HCO_3^-]; Fig. 2A, 2B) and lowered the resting arterial oxygen content (Fig. 3B), Hb_{O_2} (Fig. 3C), and MCHC. We anticipate from previous histological studies (Chevalier et al. 1985; Karlsson-Norrgren et al. 1986a, 1986b; Tietge et al. 1988; Mueller et al. 1991) that preexposure to Al would cause a thickening of the branchial water-to-blood diffusion distance. This would result from general hyperplasia and proliferation of mucus and chloride cells on the lammellar epithelium. As a consequence, despite the maintenance of Pa_{O_2} and Pa_{CO_2} under these conditions, reduced blood O_2 content and Hb_{O_2} occur probably due to Bohr and Root effects associated with the lowered plasma pH and [HCO_3^-]. In other words, Pa_{O_2} and Pa_{CO_2} are maintained but the O_2 carrying capacity, CO_2 carrying capacity, and buffer capacity of the blood are all reduced. As $\dot{M}_{O_2}$ was unaffected (Fig. 1D), either cardiac output must be greater or the venous O_2 reserve must be reduced in these Al-preexposed fish.

The challenge ultimately resulted in significant acid–base and ionic disturbances in both groups of fish as revealed in the terminal samples (Table 2). Although the responses of the Al-naive fish to Al challenge reported here were similar to those reported by Wood et al. (1988c), there were some discrepancies regarding the responses of the Al-preexposed fish, in par-

ticular the decrease in plasma [Cl^-] and the deterioration of the blood acid–base status seen here as a result of the challenge. In addition, Wood et al. (1988a, 1988c) and McDonald et al. (1991) both reported substantial improvements in survivorship of brook trout preexposed to sublethal levels of Al when challenged with a lethal combination of acid/Al. The present study demonstrated much smaller and nonsignificant differences with regard to LT50. We suspect that a major factor in these discrepancies was the combined stresses of masking and cannulation in the present study.

With regards to the original hypotheses, although we were not able to unequivocally demonstrate an increase in survivorship to Al challenge as a result of sublethal Al preexposure, some ameliorative effects were apparent, most notably the elimination of hyperventilation during severe Al challenge.

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References

AUDET, C., AND C. M. WOOD. 1988. Do rainbow trout (*Salmo gairdneri*) acclimate to low pH? Can. J. Fish. Aquat. Sci. 45: 1399–1405.

BOUTILIER, R. G., T. A. HEMING, AND G. K. IWAMA. 1984. Physicochemical parameters for use in fish respiratory physiology, p. 403–430. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology, Vol. Xa. Academic Press, Orlando, FL.

CHEVALIER, G., L. GAUTHIER, AND G. MOREAU. 1985. Histopathological and electron microscope studies of gills of brook trout, *Salvelinus fontinalis*, from acidified lakes. Can. J. Zool. 63: 2062–2070.

DAVIS, J. C., AND J. N. CAMERON. 1971. Water flow and gas exchange at the gills of rainbow trout, *Salmo gairdneri*. J. Exp. Biol. 54: 1–18.

DEJOURS, P. 1975. Principles of comparative respiratory physiology. American Elsevier, New York, NY. 253 p.

HARVEY, H., AND J. MCARDLE. 1986. Physiological responses of rainbow trout *Salmo gairdneri* exposed to plastic lake inlet and outlet stream waters. Water Air Soil Pollut. 30: 687–694.

KARLSSON-NORRGREN, L., W. DICKSON, O. LJUNGBERG, AND P. RUNN. 1986a. Acid water and aluminum exposure: gill lesions and aluminum accumulation in farmed brown trout, *Salmo trutta* L. J. Fish Dis. 9: 1–9.

1986b. Acid water and aluminum exposure: experimentally induced gill lesions in brown trout, *Salmo trutta* L. J. Fish Dis. 9: 11–25.

LAZERTE, B. D. 1984. Forms of aqueous aluminum in acidified catchments of Central Ontario: a methodological analysis. Can. J. Fish. Aquat. Sci. 41: 766–776.

LITCHFIELD, J. T. 1949. A method for rapid graphic solution of time – per cent effect curves. J. Pharmacol. Exp. Ther. 97: 399–408.

LITCHFIELD, J. T., AND F. WILCOXON. 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96: 99–113.

MALLANT, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can. J. Fish. Aquat. Sci. 42: 630–648.

MALTE, H., AND R. E. WEBER. 1988. Respiratory stress in rainbow trout dying from aluminum exposure in soft, acid water, with and without sodium chloride. Fish Physiol. Biochem. 5: 249–256.

MCDONALD, D. G., C. M. WOOD, R. G. RHEM, M. E. MUELLER, D. R. MOUNT, AND H. L. BERGMAN. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). I. Physiology. Can. J. Fish. Aquat. Sci. 48: 2006–2015.

MUELLER, M. E., D. A. SANCHEZ, H. L. BERGMAN, D. G. MCDONALD, R. G. RHEM, AND C. M. WOOD. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. Can. J. Fish. Aquat. Sci. 48: 2016–2027.

MUNIZ, I. P., AND H. LEIVESTAD. 1980. Toxic effects of aluminum on the brown trout, *Salmo trutta* L., p. 320–321. *In* D. Drablos and A. Tollan [ed.] Ecological impact of acid precipitation. SNSF project, Norway.

NEVILLE, C. M. 1985. Physiological response of juvenile rainbow trout, *Salmo gairdneri*, to acid and aluminum — prediction of field responses from laboratory data. Can. J. Fish. Aquat. Sci. 42: 2004–2019.

ORR, P. L., R. W. BRADLEY, J. B. SPRAGUE, AND N. J. HUTCHINSON. 1986. Acclimation-induced change in toxicity of aluminum to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 243–246.

PLAYLE, R. C., G. G. GOSS, AND C. M. WOOD. 1989. Physiological disturbances in rainbow trout during acid and aluminum exposures in soft water of two calcium concentrations. Can. J. Zool. 67: 314–324.

PLAYLE, R. C., AND C. M. WOOD. 1989. Water pH and aluminum chemistry in the gill micro-environment of rainbow trout during acid and aluminum exposure. J. Comp. Physiol. B 159: 539–550.

REID, S. D., D. G. MCDONALD AND R. R. RHEM. 1991. Acclimation to sublethal aluminum: modification of metal – gill surface interactions of juvenile rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 48: 1996–2005.

ROSSELAND, B. O. 1980. Physiological responses to acid water in fish. 2. Effects of acid water on metabolism and gill ventilation in brown trout, *Salmo trutta* L., and brook trout, *Salvelinus fontinalis* Mitchill, p. 348–349. *In* D. Drablos and A. Tollan [ed.] Ecological impact of acid precipitation. SNSF project, Norway.

SCHOFIELD, C. L. 1977. Acid snow-melt effects on water quality and fish survival in the Adirondack Mountains of New York State. Res. Tech. Completion Report, Project No. A-072-NY.

TIETGE, J. E., R. D. JOHNSON, AND H. L. BERGMAN. 1988. Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long-term exposure to acid and aluminum. Can. J. Fish. Aquat. Sci. 45: 1643–1648.

WALKER, R. L., C. M. WOOD, AND H. L. BERGMAN. 1988. Effects of low pH and aluminum on ventilation in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1614–1622.

WOLF, K. 1963. Physiological salines for freshwater teleosts. Prog. Fish-Cult. 25: 135–140.

WOOD, C. M., D. G. MCDONALD, C. E. BOOTH, B. P. SIMONS, C. G. INGERSOLL, AND H. L. BERGMAN. 1988a. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. 45: 1587–1596.

WOOD, C. M., R. C. PLAYLE, B. P. SIMONS, G. G. GOSS, AND D. G. MCDONALD. 1988b. Blood gases, acid–base status, ions, and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure. Can. J. Fish. Aquat. Sci. 45: 1575–1586.

WOOD, C. M., B. P. SIMONS, D. R. MOUNT, AND H. L. BERGMAN. 1988c. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 2. Blood parameters by cannulation. Can. J. Fish. Aquat. Sci. 45: 1597–1605.

ZAR, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ. 718 p.

Acclimation to Sublethal Aluminum: Modifications of Metal – Gill Surface Interactions of Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Reid, S. D., D. G. McDonald, and R. R. Rhem. 1991. Acclimation to sublethal aluminum: modifications of metal – gill surface interactions of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1996–2005.

Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed for 21 d to one of three concentrations of H^+ and Al. Exposure to 27 μg Al·L^{-1} at pH 5.2 resulted in a significant internal physiological disturbance associated with the accumulation of Al at the gill, both of which declined with continued exposure. Increased resistance to an acutely lethal Al concentration (118 μg·L^{-1}, pH 5.2) was demonstrated by a reduction in both rate of mortality and in the magnitude of the internal physiological disturbance relative to controls at pH 6.5 or 5.2 and 0 μg Al·L^{-1}. Both Al and H^+ were strong Ca^{2+} antagonists causing modifications in the ability of the gill microenvironment to bind Ca^{2+} during the acclimation exposures (saturation binding and competitive inhibition techniques used). The Al resistance was associated with a significant reduction in both the binding affinity of the gill surface for Al and the ability of Al to inhibit gill Ca^{2+} binding. This reduction in the surface activity of Al in the fish with prior Al exposure suggested that increased Al resistance involved modifications in the chemical characteristics of the anionic groups present in the gill microenvironment, resulting in the reestablishment of membrane stability and electrolyte permeability.

On a exposé des truites arc-en-ciel (*Oncorhynchus mykiss*) juvéniles à trois différentes concentrations de H^+ et d'Al pendant 21 jours. L'exposition à 27 μg Al·L^{-1} en milieu de pH 5,2 a entraîné une perturbation significative de la physiologie interne liée à l'accumulation d'Al dans les branchies. Ces deux phénomènes ont diminué d'ampleur en exposition continue. Une baisse du taux de mortalité et de l'ampleur de la perturbation de la physiologie interne par rapport aux poissons témoins exposés à 0 μg Al·L^{-1} en milieu de pH 5,2 et 6,5 a prouvé l'existence d'une résistance accrue à une concentration létale aiguë d'Al (118 μg·L^{-1}, pH 5,2). On a noté que Al et H^+ étaient de puissants antagonistes du Ca^{+2}, modifiant la capacité de fixation du Ca^{+2} dans le micro-environnement des branchies lors des épreuves d'acclimatation (saturation par fixation et inhibition par association exclusive). La résistance à Al était liée à une baisse significative de l'affinité de fixation du Al à la surface des branchies et à la capacité du Al d'inhiber la fixation du Ca^{+2} par les branchies. Cette baisse de l'activité de surface du Al chez les poissons pré-exposés porte à croire que la résistance augmentée au Al suppose des modifications des caractéristiques chimiques des groupes anioniques présents dans le micro-environnement des branchies, modifications qui entraînent le rétablissement de la stabilité de la membrane et de la perméabilité aux électrolytes.

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Water in regions impacted by acidic precipitation often exhibits elevated Al concentrations as a result of Al mobilization from soils and sediments (Wright and Gjessing 1976). Loss of fish populations, often attributed directly to the acidification of lakes and rivers (Harvey 1982), has been ascribed to elevated Al levels (Schofield and Trojnar 1980) in some instances. The mechanism of Al toxicity to fish is thought to involve either ionoregulatory or respiratory disturbances or both. The severity and relative contribution of each appears to be critically dependent on the water pH and the Al concentration (Neville 1985; Booth et al. 1988; Wood et al. 1988a, 1988b, 1988c). Since significant accumulation of Al on the gills has been observed (Youson and Neville 1987), with no increase in plasma Al levels (Booth et al. 1988), it has been proposed that Al is toxic to fish primarily because of its action on the gill (Schofield and Trojnar 1980; Wood et al. 1988c). One postulated toxic mechanism of Al is displacement of Ca^{2+} from membrane stabilizing sites on the gill (Booth et al. 1988, Wood et al. 1988b). Thus, exposure to Al in concert with reduced water pH is thought to lead to an impairment of ionoregulation, as a result of a decrease in gill membrane integrity. H^+ is thought to operate by a similar fashion (McDonald 1983).

One characteristic of prolonged sublethal exposure to Al is that fish develop a resistance to Al (i.e. "acclimate" to Al). Toxicant tolerance is typically assessed by performance of the animals in LC_{50} trials. The LC_{50} is the concentration of toxicant resulting in 50% mortality within a specific time. Orr et al. (1986) demonstrated that a 1- or 2-wk exposure to sublethal Al causes increased Al resistance in juvenile rainbow trout based on a near doubling of the 120-h LC_{50}. Increased resistance of fish to other metals (Cu, Cd, Zn) following exposure to sub-

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lethal concentrations has also been reported and typically results in a 1.5- to 2.7-fold increase in the observed LC_{50} (see review by Hodson 1988). In some instances, the increase in metal resistance was linked to the induction of intracellular metal-binding proteins, including cysteine-rich metallothionein. However, at least in the case of Cu, increased resistance, as indicated by the recovery of branchial Na^+ exchange in the presence of Cu, occurred without the induction of metal-binding proteins in the gills (Lauren and McDonald 1987a, 1987b).

Since Al causes ionoregulatory disturbances via its action on the gill, it is reasonable to assume that the mechanism of acclimation occurs through modifications of the gill. In brook trout (*Salvelinus fontinalis*), this gill modification appears to result in the recovery of lost electrolytes (Wood et al. 1988a), both from a recovery of Na^+ transport activity (McDonald and Milligan 1988) and a reduction in passive permeability during Al/acid challenge (Booth et al. 1988). Modification in the interaction of Al and Ca^{2+} at the level of the gill may lead to improved epithelial integrity, due to the role of Ca^{2+} in maintaining membrane stability.

The objectives of this study were to (1) document physiological disturbances in rainbow trout (*Oncorhynchus mykiss*) Al exposure, (2) investigate the time course and nature of physiological resistance (i.e. acclimation) to Al, and (3) study some of the quantitative modifications of the gills that may be associated with Al acclimation: gill sialic acid (an indicator of gill mucus content), gill Al accumulation in relation to chronic sublethal and acutely lethal Al levels, and gill Ca^{2+}-binding activity as assessed by a new in vitro method.

Materials and Methods

Animals

Experiments were conducted at the Fish Physiology and Toxicology Laboratory, University of Wyoming. Juvenile rainbow trout, (weight range 10–25 g) were obtained from Cline's Trout Farm (Boulder, CO) and were maintained prior to experimentation for at least 6 wk in a 600-L fiberglass holding tank continuously supplied with very soft water (Table 1) at a temperature of 15 ± 0.5°C. Fish were fed commercial trout chow at the rate of 1% of body weight per day and tanks were siphoned daily to remove organic debris.

Experimental Protocol

The basic experimental approach was to acclimate juvenile trout to one of three conditions (control, low pH, low pH, and Al) for 21 d. During the acclimation period, blood chemistry, gill tissue composition, and gill Ca^{2+}-binding activity were monitored. After 21 d, fish from all treatments were challenged with an acutely lethal concentration of Al. Increased Al resistance was assessed on the basis of estimated times to 50% mortality (LT_{50}'s) during challenge tests for the three acclimation groups when exposed to pH 5.2 and 118 μg $Al \cdot L^{-1}$ (Al challenge).

Al exposure levels for acclimation (30 μg $\cdot L^{-1}$, pH 5.2) and lethal challenge (100 μg $\cdot L^{-1}$, pH 5.2) were chosen on the basis of preliminary trials with various combinations of Al and/or low pH. Actual mean concentrations of Al were respectively 27 and 118 μg $\cdot L^{-1}$ (Table 1). These low pH and Al concentrations were comparable with levels measured in Canadian Shield and Adirondack lakes undergoing acidification (Schofield and Trojnar 1980; Chevalier et al. 1985).

The original fish maintained under the holding conditions are referred to as the 6.5/0 (pH 6.5 and 0 μg $Al \cdot L^{-1}$) or control fish. Those animals exposed to Al and low pH during the acclimation period are referred to as the 5.2/27 (pH 5.2 and 27 μg $Al \cdot L^{-1}$) or Al fish, while fish that were exposed to low pH alone (pH 5.2 and 0 μg $Al \cdot L^{-1}$) are referred to as the low-pH or 5.2/0 fish.

Acclimation Studies

At the start (day 0), 165 fish were removed from the control tank. Each was killed with a single, sharp blow to the head. Blood samples were taken from five fish by caudal puncture with a heparinized syringe and processed immediately for hematocrit, plasma Cl^-, and plasma protein. The branchial baskets of these five fish were carefully removed, weighed, and digested in 3.5 volumes of 0.1 N nitric acid for later determination of gill Al and sialic acid content. From the remaining 160 fish, whole branchial baskets were removed, rinsed for 15 s in each of distilled water, 5.0 mM EDTA, and distilled water, and then used immediately for the determination of the half-saturation time ($t_{1/2}$, $N = 80$) or gill binding capacity (B_{max}) and apparent dissociation constant (K_D, $N = 80$) for Ca^{2+}.

Approximately 1300 6.5/0 fish were then transferred and maintained at either pH 5.2 alone or pH 5.2 and 27 μg $Al \cdot L^{-1}$ for 21 d, while an additional 700 fish remained in the control (6.5/0) tank. Two 350-L tanks were used for the 5.2/0 and 5.2/27 treatments with the fish divided equally amongst the four tanks. Each tank was supplied continuously with soft water of the appropriate water chemistry (Table 1) at a temperature of 15 ± 0.5°C. Feeding of fish continued during the 21-d exposures at 1% of body weight per day using commercial trout chow. Tanks were siphoned daily to remove organic debris.

At days 8 and 21, 165 fish were again removed from the three exposure conditions (6.5/0, 5.2/0, 5.2/27). Blood and

TABLE 1. Composition and pH (means, SEM in parentheses) of the test water. Water samples were taken throughout the exposure period for determination of water chemistry. $N = 12$; temperature = 15 + 0.5°C.

	pH	Ca^{2+} (μequiv·L^{-1})	Total Al (μg·L^{-1})	Na^+ (μequiv·L^{-1})	Mg^{2+} (μequiv·L^{-1})	K^+ (μequiv·L^{-1})	F^- (μequiv·L^{-1})	Cl^- (μequiv·L^{-1})	NO_3^- (μequiv·L^{-1})	SO_4^{2-} (μequiv·L^{-1})
Control	6.48	32.6	0.3	43.3	21.2	77.5	1.0	44.3	1.4	33
(6.5/0)	(0.04)	(1.78)	(0.16)	(1.79)	(16.4)	(18.8)	(0.09)	(0.78)	(0.14)	(16.4)
Low pH	5.19	34.0	0.2	45.2	21.6	77.9	1.2	46.4	1.4	65.7
(5.2/0)	(0.06)	(1.02)	(0.09)	(0.85)	(0.36)	(18.8)	(0.08)	(1.08)	(0.20)	(23.6)
Low pH/Al	5.12	27.4	26.6	44.6	20.8	78.3	1.0	48.6	1.2	126.8
(5.2/27)	(0.06)	(0.84)	(3.12)	(1.01)	(0.38)	(18.4)	(0.09)	(1.39)	(0.18)	(20.8)
Al challenge	5.17	30.2	117.7	43.0	21.6	3.3	3.0	54.5	2.2	57.8
	(0.06)	(0.44)	(3.30)	(0.91)	(0.28)	(0.39)	(0.10)	(0.38)	(0.32)	(0.02)

branchial baskets were sampled from five fish in each group and the remainder were utilized in the measurements of gill Ca^{2+}-binding activity. Also on day 21, the effect of H^+ or Al (at pH 5.2) on gill Ca^{2+} binding was determined on an additional 160 fish from each group. Half of these fish ($N=80$) were used to determine the effect of Al (pH 5.2) on Ca^{2+}-binding activity (Al IC_{50}), while the remainder ($N=80$) were used to determine the effect of H^+ on Ca^{2+}-binding activity (H^+ IC_{50})⟩

Lethal Al Challenge

On day 21, fish from each acclimation condition (6.5/0, 5.2/0, 5.2/27, $N=20$ from each) were transferred to separate challenge tanks. Each tank was supplied with soft water at pH 5.2 containing 118 μg Al·L^{-1} at a temperature of 15 ± 0.5°C (Table 1). Mortality in these tanks was monitored approximately every 2 h for 70 h, with dead fish being removed immediately upon discovery. After 40 h, five survivors from each treatment group were removed from the challenge tanks and their blood hematocrit, plasma Cl^-, plasma protein, and gill Al were measured.

Analytical Methods

Blood

Hematocrit was read directly from heparinized microhematocrit tubes spun for 3 min at 9000 × *g*. Protein concentration in 8 μL of plasma was determined with an American Optical Goldberg refractometer (Alexander and Ingram 1980). Plasma Cl^- was determined on a 20-μL sample by coulometric titration using a Radiometer CMT10.

Gill sialic acid content

Mucus secreted by the gills of trout is rich in sialoglycoprotein (Fletcher et al. 1976). Therefore, we used a method of analyzing the sialic acid content of the gill as an indicator of the gill mucus content. Gill sialic acid content was assayed on 25-μL samples of gill tissue digest according to the thiobarbituric acid assay of Warren (1959, 1963). Sialic acid samples were prepared in triplicate and measured against known standards (sialic acid = *N*-acetylneuraminic acid, Sigma A2751) on a UV/VIS spectrophotometer (Philips PU 8600).

Gill Al

Gill Al was assayed in triplicate on 50 μL of gill tissue digest added to 3.5 mL of 0.1 N HCl using a modified version of the pyrocatechol violet method of Dougan and Wilson (1974). To account for colourimetric interference from the tissue digest itself, 50 μL of tissue digest from 6.5/0 fish was added to all standards and blanks. Absolute gill content of Al may be underestimated by the accumulation of trace Al in the 6.5/0 water, and thus, the measurements were considered relative to controls (6.5/0).

Calcium binding activity

Separate experiments were performed to determine (i) the half-saturation time ($t_{1/2}$) of gill Ca^{2+} binding, (ii) the apparent dissociation constant (K_d) and maximum capacity (B_{max}) of gill Ca^{2+} binding, and (iii) the ability of Al (at pH 5.2) or of H^+ to interfere with gill Ca^{2+} binding (IC_{50}).

Following removal and rinsing of the isolated branchial baskets, the branchial baskets were exposed immediately to radiocalcium (^{45}Ca) solutions for various lengths of time dependent on the parameter of interest. All binding assays were performed on individual branchial baskets in 50 mL of assay solution in a 125-mL sample cup. Each sample cup was fitted with an air line to provide aeration and mixing and placed in a 15 ± 0.5°C water bath.

(i) $t_{1/2}$ — Isolated branchial baskets were exposed to 10^{-2} M ^{45}Ca (New England Nuclear) for one of nine durations ranging from 15 to 1800 s ($N=4$). The specific activity of the ^{45}Ca solution was approximately 18 kBq·μmol^{-1}.

(ii) K_D and B_{max} — Branchial baskets were exposed to one of nine ^{45}Ca concentrations ranging from 10^{-7} to 10^{-2} M Ca^{2+} for exactly 300 s. Over the range of Ca^{2+} concentrations, the specific activity was kept constant at 18.5 kBq·μmol^{-1}.

(iii) IC_{50} — Branchial baskets were exposed to one to nine solutions of constant Ca^{2+} concentration (0.5 mM) and inhibitor concentrations ranging from 10^{-6} to 10^{-2} M H^+ or 3.7 × 10^{-6} to 3.7 × 10^{-5} M (100–1000 μg·L^{-1}) Al (pH 5.2) for 300 s. The specific activity of the ^{45}Ca was kept constant at approximately 18.3 kBq·μmol^{-1}.

At the end of the binding activity exposures, branchial baskets were rinsed in distilled water for 15 s to removed excess assay solution, placed in preweighed 20-mL scintillation vials, weighed, and digested in 3.5 volumes of 0.1 N nitric acid overnight.

^{45}Ca activity was determined on 100 μL of gill digest and on 5 mL of assay solution taken immediately before and after the gill exposures. Each sample was added to 10 mL of scintillation fluor (ACS, Amersham), with 4.9 mL of water added to the gill digests, and each was counted for at least 20 min on a liquid scintillation counter (Beckman LS 9000). Each sample was prepared in triplicate and all samples were corrected for quench according to a quench curve generated with counting samples containing a known amount of radioactivity and varied quantities of gill digest.

Measurements of gill Ca^{2+} binding were analyzed using nonlinear regression (Johnston 1985) and Scatchard lot techniques (Scatchard 1949). Half-saturation time ($t_{1/2}$) was calculated from the analysis of the saturation of the tissue as a function of time, using nonlinear regression (Johnston 1985). A Scatchard plot is the graph generated by the following equation:

$$(1)\quad \frac{(D^*G)}{(D^*)} = -\frac{(D^*G)}{(K_{D^*})} + \frac{(D^*G)_{max}}{(K_{D^*})}$$

where, for our application, D^* is the free Ca concentration (Free), D^*G is the amount of Ca^{2+} bound (Bound) to the gill (G), K_{D^*} is the apparent dissociation constant of the binding of Ca^{2+} to the gill, and D^*G_{max} represents the total binding capacity (B_{max}) of the gill. By varying the amount of Ca in the radiocalcium assay solution and plotting Bound/Free against the amount Bound, the K_D and B_{max} are determined from the slope and x-intercept of the Scatchard plot, respectively.

The inhibition curves (% Ca^{2+} bound versus inhibitor concentration) were linearized using the ln-logit transformation according to Sandor et al. (1984):

$$(2)\quad \text{Ln-logit} = \text{Ln}(\text{Bound}/(B_{max} - \text{Bound}))$$

where Bound is the amount of bound Ca^{2+} in the presence of the inhibitor (H^+ and/or Al), while B_{max} is the binding capacity, for a particular treatment group, in the absence of the inhibitor. The IC_{50} values, or the concentration of inhibitor resulting in a 50% reduction in the Ca^{2+}-binding capacity, was taken as the concentration of inhibitor at a ln-logit value of zero (see Fig. 7C).

Apparent dissociation constants (K_D) for H^+ and Al binding were determined indirectly according to the equation of Cheng and Prusoff (1973):

$$(3) \quad K_D = IC_{50} \cdot (1 + (D^*/K_{D^*}))^{-1}$$

using the estimated IC_{50} for the appropriate antagonist, the apparent Ca^{2+} dissociation constant of the specific treatment group (K_{D^*}), and the concentration of ^{45}Ca in the assay solution (D^*).

Ca^{2+} and Al Concentrations

Ca^{2+} concentration in acclimation tanks, challenge tanks, and the Ca^{2+}-binding activity assay solutions was measured in duplicate against known standards by atomic absorption flame spectrophotometry (Perkin-Elmer model 2380). Al content of the Ca^{2+}/Al assay solutions used in the competitive inhibition assays was measured in triplicate samples of 3.5 mL using a modified version of the pyrocatechol violet method of Dougan and Wilson (1974). Acclimation and challenge tank Al levels were measured on the Perkin-Elmer AA with an associated high-temperature graphite furnace (Perkin-Elmer model HGA-400).

Statistics

Data are reported as means ± 1 SEM. Differences between means and specific binding characteristics were determined using a two-tailed, unpaired Student's *t*-test with significance set at a 95% level of confidence. Survival curves were compared using standard log/probit analysis and nomographic methods to determine median survival times (LT_{50}), their 95% confidence limits, slope functions, and the significance of differences between treatments (Litchfield 1949; Litchfield and Wilcoxon 1949).

Results

Aluminum Acclimation

Blood chemistry

Plasma Cl^- in fish held at 6.5/0 (controls) did not change significantly from an overall mean concentration of 12.67 ± 1.41 mequiv·L^{-1} (Fig. 1A). Exposure to pH 5.2 soft water (5.2/0) resulted in only minor changes in Cl^-, with only the increase at day 21 being statistically significant. A severe reduction in Cl^- resulted from the exposure of juvenile trout to Al and low pH (5.2/27). A 24% decline in Cl^- was measured after 8 d of exposure to 27 μg Al·L^{-1} at pH 5.2 (5.2/27) and Cl^- remained significantly lower than both control and low pH only levels, although some recovery of Cl^- was noted at day 21.

No significant changes were noted in hematocrit or protein concentration in the 6.5/0 group, although both tended to decline over the 21-d period (Fig. 1B, 1C). As was the case for Cl^-, only minor differences between the 6.5/0 and 5.2/0 estimates of protein and hematocrit were evident (Fig. 1C). In contrast, protein and hematocrit were elevated 34.2 and 12.5%, respectively, in the 5.2/27 group at day 8 compared with controls. Increased hematocrit and plasma protein is usually taken as evidence of hemoconcentration resulting from loss of electrolytes (Milligan and Wood 1982). By day 21, some recovery was evident, as hematocrit was no longer significantly different from either 6.5/0 or 5.2/0 levels. In addition, the protein concentration of the 5.2/27 fish was the same as the 5.2/0 fish while still remaining significantly greater than the protein concentration of the 6.5/0 fish due to a minor reduction in the day 21 measure.

Gills

Al exposure (27 μg·L^{-1}) resulted in a significant accumulation of Al. By day 8, gills of the 5.2/27 fish contained 25.6 ± 1.47 μg·g^{-1}, with a significant reduction in gill Al occurring by day 21 (Fig. 2A). In contrast, gill sialic acid content remained virtually constant in all three groups (Fig. 2B).

Al Challenge

Mortality

Prior exposure to Al (27 μg·L^{-1}, pH 5.2) but not to pH 5.2 alone, significantly reduced mortality to the 118 μg·L^{-1} (pH 5.2) Al challenge (Fig. 3). The LT_{50}'s for the 6.5/0 and 5.2/0 fish were 36.0 and 31.5 h, respectively, and not significantly different from one another. At this time (30–40 h) the mortality of the 5.2/27 fish was only 5%.

Blood chemistry

Little difference was noted in the blood chemistry between the 6.5/0 and 5.2/0 fish when challenged with 118 μg Al·L^{-1} at pH 5.2 for 40 h (Fig. 1). Both groups experienced significant reductions in Cl^- (Fig. 1A) and elevations in both hematocrit (Fig. 1B) and plasma protein (Fig. 1C), although the rise in 5.2/0 hematocrit was significantly lower than in the controls. The increased survival of the 5.2/27 trout was reflected in a significantly lower plasma Cl^- loss and a correspondingly smaller hematological disturbance, compared with controls (Fig. 1).

Gills

The gills of fish from all treatment groups (6.5/0, 5.2/0, 5.2/27) accumulated Al (Fig. 2A). In the 6.5/0 and 5.2/0 groups, this amounted to 190 ± 24.0 and 172 ± 18.0 μg·g^{-1}, respectively, whereas the additional Al accumulation of the 5.2/27 fish was approximately 50% less (81.7 ± 9.1 μg·g^{-1}).

Ca^{2+} Binding Activity

Ca^{2+} saturation kinetics

The half-saturation time ($t_{1/2}$) for Ca^{2+} binding of the 6.5/0 gill tissue was 179 ± 11.5 s (Fig. 4) and did not vary significantly with either time or treatment (i.e. 5.2/0, 5.2/27). K_D of controls was 0.66 ± 0.058 mM with a B_{max} of 0.93 ± 0.082 μmol·g^{-1} on day 0 and also did not change significantly over the course of the experiment (Fig. 5). The Ca^{2+} binding activity of these softwater acclimated fish was not significantly different from that measured using similarly acclimated juvenile rainbow trout completed at McMaster University prior to this study (Reid 1989).

B_{max} of the 5.2/0 gills declined with time and was found to be significantly lower than the controls by day 21 (Fig. 6A). B_{max} of the 5.2/27 gills declined more rapidly, but by day 21 had recovered to control levels (Fig. 6A). Exposure to both 5.2/0 and 5.2/27 led to a significant increase in the Ca^{2+}-binding affinity (reduction in K_D) when compared with controls (Fig. 6B) on day 8. Further exposure had no further effect on the affinity of the 5.2/0 fish, whereas in the 5.2/27 fish the affinity had increased to 1.4 times control by day 21.

Competitive inhibition experiments

At day 21, increasing the concentration of H^+ or Al in the presence of constant Ca^{2+} resulted in a decrease in gill surface

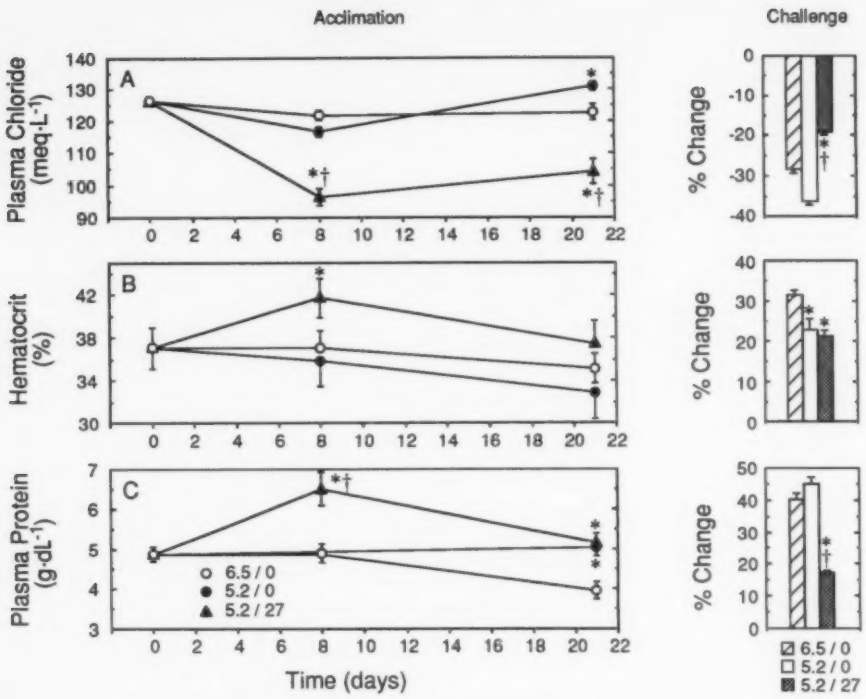

FIG. 1. (A) Plasma Cl^-, (B) plasma protein, and (C) hematocrit of juvenile rainbow trout during a 21-d acclimation exposure to softwater at pH 6.5 (6.5/0, open circles), pH 5.2 (5.2/0, closed circles), or 27 μg $Al \cdot L^{-1}$ at pH 5.2 (5.2/27, closed triangles). Panels to the right depict percent change in the day 21 values after 40 h of challenge exposure to 118 μg $Al \cdot L^{-1}$ at pH 5.2. Data presented as means ± 1 SEM; $N = 5$ for each treatment. Significantly different means (two-tailed Student's t-test, $p \leq 0.05$) indicated by an asterisk or dagger for comparisons with control (6.5/0) or low pH only (5.2/0), respectively.

Ca^{2+} binding (Fig. 7A, 7B) in all three treatment groups. The IC_{50}'s for H^+ of gills from fish with prior low pH exposure (5.2/0, 5.2/27) were significantly lower than controls, although not significantly lower than each other, indicating an increase in the ability of H^+ to inhibit gill Ca^{2+} binding. The Al IC_{50}'s were up to an order of magnitude lower than the IC_{50}'s for H^+ (Fig. 7D), suggesting greater inhibition of Ca^{2+} binding by Al than H^+. Twenty-one days of exposure to both 5.2/0 and 5.2/27 resulted in a significant increase in the Al IC_{50} compared with controls, indicating a decrease in the ability of Al to inhibit gill Ca^{2+} binding. The effectiveness of Al as a Ca^{2+} antagonist in the 5.2/0 group was reduced 13% and was reduced further, almost 80%, in the 5.2/27 fish.

The calculation of the K_D's for Al and H^+ (Fig. 8) shows that the gill surface has the highest affinity for Al, then H^+, followed by Ca^{2+}, in the ratio of 46:3:1 (based on the K_D's for controls). This ranking did not change with treatment, but the relative ratios did vary, resulting in ratios of 28:8:1 for the low-pH (5.2/0) fish and 16:10:1 for thet Al (5.2/27) fish. The relative affinity ratios suggest that chronic exposure to low pH results in an increase in the gill's affinity for H^+ with respect to Ca^{2+} and Al whereas chronic exposure to low pH and Al results in a decrease in the affinity for Al relative to Ca^{2+}.

Discussion

This study shows that exposure of rainbow trout to Al and low pH results in disturbances in branchial ionoregulation similar to those recently described in detail for brook trout (Booth et al. 1988; McDonald and Milligan 1988; Wood et al. 1988b; McDonald et al. 1991). However, rainbow trout are clearly more sensitive to Al and low pH than brook trout. For example, no Al-dependent ionoregulatory disturbances develop in brook trout exposed to 75 μg $Al \cdot L^{-1}$ at pH 5.2 (McDonald et al. 1991), while in this study, under comparable conditions, significant Al-dependent physiological disturbances occur in rainbow trout at only 27 μg $Al \cdot L^{-1}$. In addition, rainbow trout at 27 μg $Al \cdot L^{-1}$ accumulate as much gill Al as do brook trout at an almost threefold greater Al concentration (McDonald et al. 1991).

This study also confirms that juvenile rainbow trout develop increased Al resistance specifically due to sublethal Al exposure (Orr et al. 1986), and not due to the accompanying low pH (Audet and Wood 1988). The 5.2/27 fish performed well in the Al challenge (118 μg $Al \cdot L^{-1}$), while both the 5.2/0 and 6.5/0 fish were equally adversely affected, exhibiting 50% mortality within 35 h. Finally, characterization of the gill microenvironment according to its cation-binding properties has contributed new information to the understanding of both the specific mechanism of Al toxicity and the physiological/biochemical basis for the development of Al resistance.

Gill Microenvironment and Cation Binding

In this study, we have shown that the gill microenvironment, defined as the apical epithelial surface and its external mucous

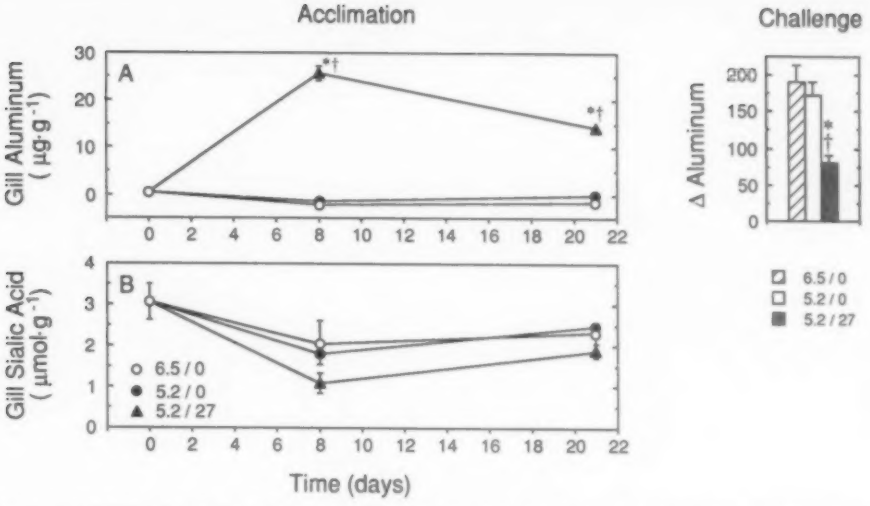

FIG. 2. (A) Gill Al and (B) sialic acid content of juvenile rainbow trout during a 21-d acclimation exposure to softwater at pH 6.5 (6.5/0, open circles), pH 5.2 (5.2/0, closed circles), or 27 μg $Al \cdot L^{-1}$ at pH 5.2 (5.2/27, closed triangles). The panel to the right depicts the change in the day 21 values after 40 h of challenge exposure to 118 μg $Al \cdot L^{-1}$ at pH 5.2. Data presented as means ± 1 SEM; $N = 5$ for each treatment. Significantly different means (two-tailed Student's t-test, $p \leq 0.05$) indicated by an asterisk or dagger for comparisons with control (6.5/0) or low pH only (5.2/0), respectively.

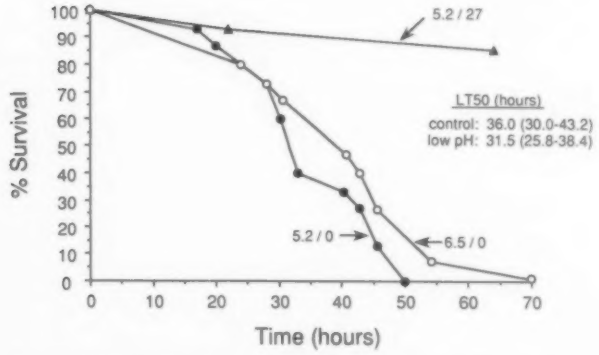

FIG. 3. Percent survival of fish from all three treatment groups (6.5/0, 5.2/0, 5.2/27) during a 188 μg $Al \cdot L^{-1}$ (pH 5.2) challenge following the 21-d acclimation exposures. Initial sample size was 20 for all treatment groups. LT_{50}'s (range) were calculated and compared using standard log/probit analysis.

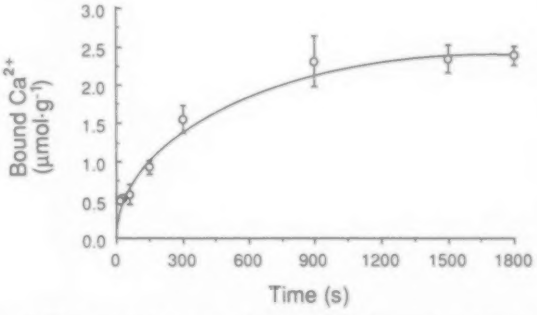

FIG. 4. Time saturation curve of gill baskets of control (6.5/0) trout. Gill baskets were removed, cleaned, and then exposed to 10^{-2} M Ca^{2+} for one of several periods of time (see Materials and methods for details). The curve was fitted to the data by eye. Data presented as means ± 1 SEM; $N = 4$.

layer, has quantifiable and reproducible cation-binding characteristics (Fig. 5). The binding properties of this region are probably due, in large part, to sulphate and carboxylate groups of acid sugars (Wold and Selset 1977; Van de Winkel et al. 1986) with pK's ranging from 2.6 to 4.0 (Dawson et al. 1986). Essential cations, such as Ca^{2+}, Na^+, and K^+ (Marshall 1978; McWilliams 1983), and trace metals (e.g. Hg^{2+} and Cd^{2+}; Part and Lock 1983) have been shown to bind to these anionic groups. Pagenkopf (1983), in developing his Gill Surface Interaction Model (GSIM), recognized the cation-binding properties of the gill as a mechanism for explaining variations in trace metal toxicity in fish. In his view, toxicity resulted from the binding of a metal to anionic groups on the gills, with toxicity being dependent not only on metal-ligand chemistry, but also on competition from hardness cations (Ca^{2+} and Mg^{2+}). Thus, Ca^{2+} has a critical role to play in gill physiology as it would act to limit trace metal binding. In addition, many studies suggest that Ca^{2+} acts to stabilize membranes through cross-binding of intercellular ligands (Williams 1979), thereby limiting overall electrolyte and nonelectrolyte permeability (Cuthbert and Maetz 1972; Eddy 1975; Ogasawara and Hirano 1984).

Toxic Mechanism of Al/H^+

Exposure of trout to H^+ or Al results in an increase in the passive electrolyte loss across the gill (McDonald 1983; Booth et al. 1988). The increase in electrolyte permeability is thought to occur, at least in part, by the displacement of membrane-bound Ca^{2+} by H^+ and Al (McDonald 1983; Booth et al. 1988). While this hypothesis is not yet proven, we can now at

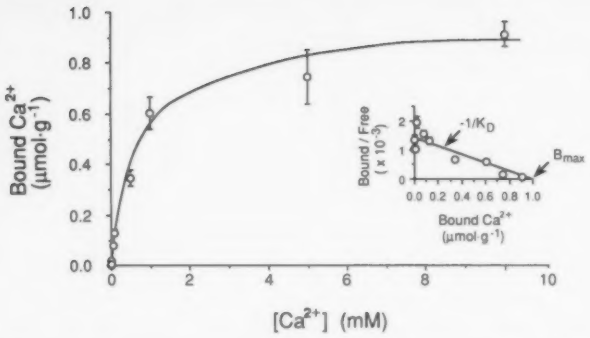

FIG. 5. Concentration saturation curve for isolated gills of the 6.5/0 fish. Gill baskets were removed, cleaned, and then exposed to a range of Ca^{2+} concentrations for exactly 300 s (see Materials and Methods for details). The curve was fitted to the data by eye. Data presented as means ± 1 SEM; $N = 4$. Insert : Scatchard plot generated from this saturation curve from which estimates of the Ca^{2+} apparent dissociation constant (K_D) and the Ca^{2+}-binding capacity (B_{max}) were determined. The line was fitted to the Scatchard plot by linear regression. Data presented as means ± 1 SEM; $N = 4$.

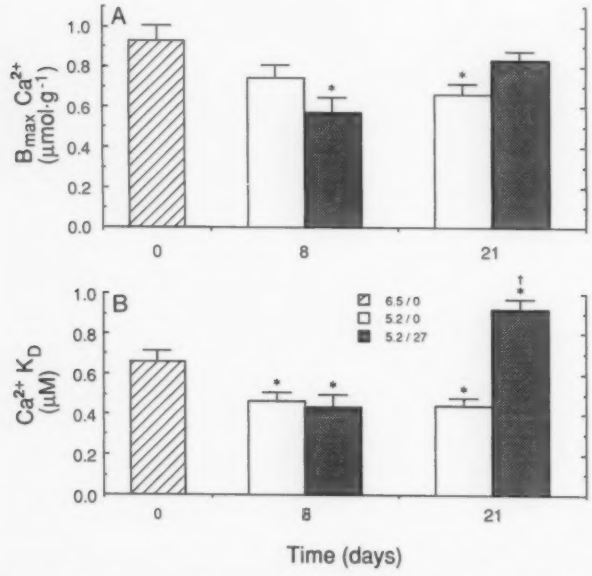

FIG. 6. Time course of changes in Ca^{2+}-binding activity. Changes in the Ca^{2+}-binding characteristics of isolated gill baskets were measured in the three treatment groups (6.5/0, 5.2/0, 5.2/27) during the 21-d acclimation period. (A) B_{max} and (B) K_D were derived from the concentration saturation curves at day 0 (control), day 8, and day 21 of exposure. Data presented as means ± 1 SEM; $N = 2$. Significantly different means (two-tailed Student's t-test, $p \leq 0.05$) indicated by an asterisk or dagger for comparisons with control (6.5/0) or low pH only (5.2/0), respectively.

least confirm that Ca^{2+}-binding sites are highly sensitive to titration by Al, having a 46-fold higher affinity for Al than Ca^{2+} at pH 5.2 (Fig. 8). At 27 µg $Al \cdot L^{-1}$ (the acclimation Al level) the Ca^{2+}/Al concentration ratio would be only 14:1 (Fig. 8); therefore, one can expect significant displacement of Ca^{2+} from binding sites. This is supported by the finding of significant initial accumulation of Al on the gills, which represents a 948-fold concentration factor over Al in the aqueous phase (25.6 µg·g^{-1} versus 0.027 µg·mL^{-1}). In addition to Al/Ca^{2+} interactions, H^+/Ca^{2+} interactions also occur (Fig. 7). The H^+ IC_{50} for the Ca^{2+}-binding groups was 3.4, i.e. an external pH of 3.4 would be required for 50% displacement of Ca^{2+}. This suggests that at the moderately acid pH of 5.2, only minor physiological impact would be expected, since little or no Ca^{2+}/H^+ competition would occur at this pH. Indeed, only a minor physiological disturbance was noted in fish maintained at 5.2/0 (Fig. 1).

Mechanism of Al Acclimation

Quite clearly, Al acclimation results in the reduction of the surface activity of Al. At least two distinct mechanisms can be suggested to explain this phenomenon: (i) an increase in mucous layer thickness on the gills or (ii) biochemical modifications to the binding ligands.

Mucification of the external gill surface has often been shown to occur in response to environmental toxicants (Mallatt 1985) and, at least in some cases, specifically to Al (Muniz and Leivestad 1980; Schofield and Trojnar 1980). Part and Lock (1983) have argued that mucus plays a critical role in protecting the gills from the effects of toxic trace metals and supported this view by showing that mucous solutions, obtained from rainbow trout, would significantly reduce the diffusion of trace metals (Cd^{2+} and Hg^{2+}) in vitro when compared with rates measured in buffer solutions. However, the weight of evidence tends to argue against this simplistic view, at least in the case of Al. Firstly, Al exposure is not always accompanied by a mucous response. For example, Karlsson-Norrgren et al. (1986) found abnormal accumulations of mucus on the surface of gills only occasionally in one of two groups of farmed brown trout (*Salmo trutta*) from acid-susceptible lakes with elevated concentrations of Al (200–300 µg·L^{-1}). In addition, Chevalier et al. (1985) showed that gills of brook trout inhabiting acidified lakes exhibited no abnormal morphology, while a whole spectrum of morphological modifications were evident in a large number of chloride cells. Secondly, one would expect that an increase in the mucous layer should be accompanied by an increase in cation-binding capacity, in gill sialic acid content, and in Al content with time. The present study, in contrast, shows no further increase in B_{max} in the 5.2/27 fish beyond that of the controls (Fig. 6), no change in the gill sialic acid content during the acclimation exposures (Fig. 3B), and, in fact, a significant decrease in the gill Al content of the 5.2/27 fish following the initial accumulation (Fig. 3A). Finally, it is unclear as to how a thicker mucous layer, simply containing more anionic groups with the same chemical characteristics, could result in differential changes in Al- and Ca^{2+} binding activity. Therefore, qualitative changes in the mucus and membrane constituents responsible for cation binding is the more probable mechanism.

A number of studies have now shown that the external epithelium of fish can respond to environmental change with qualitative alterations in ligand chemistry. Flik et al. (1984) showed that skin mucus of tilapia (*Oreochromis mossambicus*) responded to prolonged low Ca^{2+} exposure (1 mo at 0.2 mM Ca^{2+}) with a 2.3-fold increase in calmodulin, a Ca^{2+} specific protein, over that from fish maintained in hard water (0.8 mM Ca^{2+}). Furthermore, Van de Winkel et al. (1986) reported, with an identical treatment regime, a 3.2-fold increase in the glycoproteins, chondroitin sulfate and hyaluronic acid, in

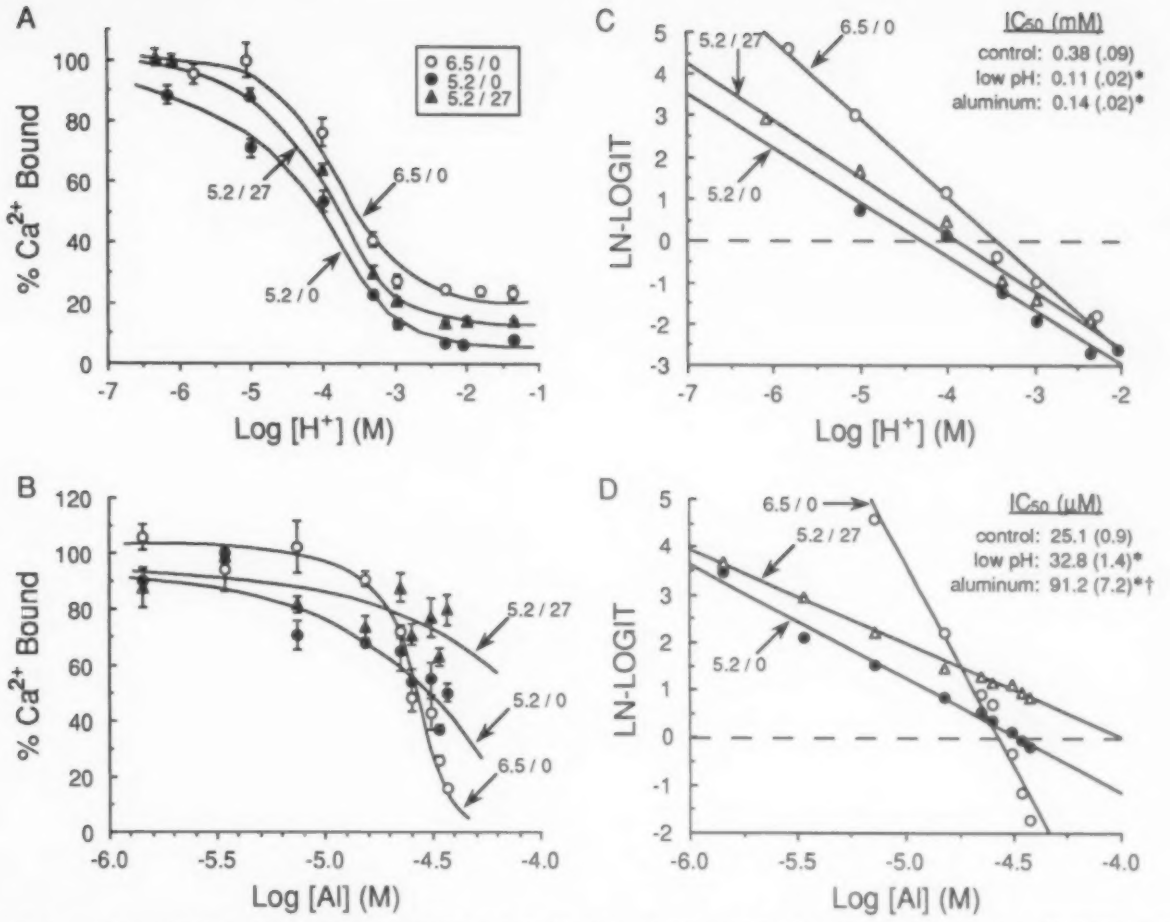

FIG. 7. Competitive inhibition of Ca^{2+} binding by (A) H^+ and (B) Al prior to (6.5/0) and following chronic exposure to low pH (5.2/0) or Al in combination with low pH (5.2/27). Curves were fitted to the data by eye. Data presented as means ± 1 SEM; $N = 4$. Competitive inhibition curves were linearized for the calculation of the (C) H^+ and (D) Al IC_{50}'s according to equation 2. Lines fitted to the linearized inhibiton curves by least squares linear regression. The IC_{50}'s, calculated from the obtained linear regression, are means ± 1 SEM from the inhibition experiments run in duplicate ($N = 2$). Significantly different means (two-tailed Student's t-test, $p \leq 0.05$) indicated by an asterisk or dagger for comparisons with control (6.5/0) or low pH only (5.2/0), respectively.

mucus removed from the body surface of rainbow trout. In addition, Zuchelkowski et al. (1985) found, based on histochemical and autoradiographic analyses of skin mucocytes of catfish (*Ictalurus nebulosus*), an apparent shift towards increased sulfomucin production in fish exposed to pH 4.8 and 4.0 compared with controls (pH 6.8). In a previous study (Reid 1989), with techniques identical to those of the present study, it was demonstrated that low $[Ca^{2+}]$ acclimation in rainbow trout is also accompanied by significant alterations in cation-binding activity of the gills. After 8 wk at 0.02 mM external $[Ca^{2+}]$, gills of juvenile trout showed a 1.64-fold increase in B_{max} and a 1.4-fold increase in Ca^{2+}-binding affinity compared with gills of trout in hard water (0.9 mM Ca^{2+}).

Clearly, therefore, there is evidence that fish can respond dynamically to environmental stress with changes in the surface chemistry of the gills. This study shows a response somewhat different from that accompanying softwater acclimation. Cation-binding capacity did not increase; instead it decreased slightly by day 8 and then recovered, but only to control levels by day 21 (Fig. 6). This suggests that rather than an increase in the quantity of various anionic groups per se, there was a change in the chemical characteristics of those groups. These changes could have occurred in the mucous layer or at the external membrane. Arguably, changes in the latter would be more important, for it is metal interactions here which have direct toxic impact. Biochemically, the gill microenvironment is very poorly described, but it is obviously highly complex. The types of changes that could occur might possibly include any one or more of the following: (i) chemical changes in membrane lipids, phospholipids, glycolipids, proteins, and glycoproteins, (ii) increased rates of membrane turnover, and (iii) incorporation or exclusion of membrane components. Quite clearly, a detailed biochemical analysis of the gills of fish, similar to that of Bolis et al. (1984), who studied gill membrane lipids of brown during acute acid exposure, would be a fruitful way of more accurately defining the exact character of the response underlying the acclimation of the gills to environmental stress.

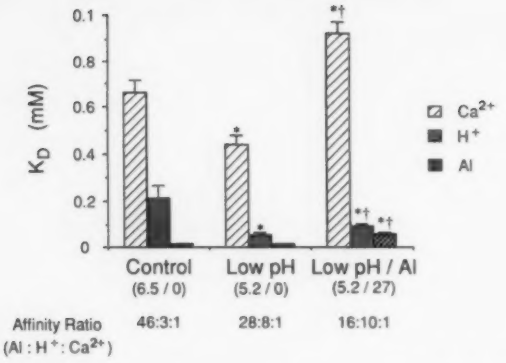

FIG. 8. Apparent dissociation constants for the binding of Ca^{2+}, H^+, and Al to the gill microenvironment. The K_D's for H^+ and Al were calculated according to equation 3 of the Materials and Methods. Affinity ratio was calculated using the inverse of the calculated K_D estimates of the gills for the appropriate cation. Data presented as means ± 1 SEM. Significantly different means (two-tailed Student's *t*-test, $p \leq 0.05$) indicated by an asterisk or dagger for comparisons with control (6.5/0) or low pH only (5.2/0), respectively.

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References

ALEXANDER, J. B., AND G. A. INGRAM. 1980. A comparison of five of the methods commonly used to measure protein concentrations in fish sera. J. Fish Biol. 16: 115–122.

AUDET, C., AND C. M. WOOD. 1988. Do rainbow trout (*Salmo gairdneri*) acclimate to low pH? Can. J. Fish. Aquat. Sci. 45: 1399–1405.

BOLIS, C. L., A. CAMBRIA, AND M. FAMA. 1984. Effects of acid stress on fish gills, p. 122–129. *In* C. L., Bolis, A. Cambria, and M. Fama [ed.] Toxins, drugs, and pollutants in marine animals. Springer-Verlag, Berlin.

BOOTH, C. E., D. G. MCDONALD, B. P. SIMONS, AND C. M. WOOD. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1563–1574.

CHENG, Y-C., AND W. H. PRUSOFF. 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22: 3099–3108.

CHEVALIER, G., L. GAUTHIER, AND G. MOREAU. 1985. Histopathological and electron microscopic studies of gills of brook trout, *Salvelinus fontinalis*, from acidified lakes. Can. J. Zool. 63: 2062–2070.

CUTHBERT, A. W., AND J. MAETZ. 1972. The effects of calcium and magnesium on sodium fluxes through the gills of *Carassius auratus*, L. J. Physiol. 221: 633–643.

DAWSON, R. M. C., D. C. ELLIOT, W. H. ELLIOT, AND K. M. JONES. 1986. Data for biological research. Oxford University Press, New York, NY. 580 p.

DOUGAN, W. K., AND A. L. WILSON. 1974. The absorptiometric determination of aluminum in water. A comparison of some chromogenic reagents and development of an improved method. Analyst 99: 413–430.

EDDY, F. B. 1975. The effect of calcium on gill potentials and on sodium and chloride fluxes in the goldfish, *Carassius auratus*. J. Comp. Physiol. 96: 131–142.

FLETCHER, T. C., R. JONES, AND L. REID. 1976. Identification of glycoproteins in goblet cells of epidermis and gill of plaice (*Pleuronectes latessa* L.), flounder (*Platichthys flesus* L.) and rainbow trout (*Salmo gairdneri* Richardson). Histochem. J. 8: 597–608.

FLIK, G., J. H. VAN RIJS, AND S. E. WENDELAAR-BONGA. 1984. Evidence for the presence of calmodulin in fish mucus. Eur. J. Biochem. 138: 651–654.

HARVEY, H. H. 1982. Population responses of fishes in acidified waters, p. 227–242. *In* R. E. Johnson [ed.] Acid Rain/Fisheries. Proceedings of an International Symposium on Acidic Precipitation and Fishery Impacts in North-eastern North America. Cornell University, Ithaca, NY, 2–5 August 1981. American Fisheries Society, Bethesda, MD.

HODSON, P. V. 1988. The effect of metal metabolism on uptake, disposition and toxicity in fish. Aquat. Toxicol. 11: 3–18.

JOHNSTON, A. 1985. SIMP: a computer program in BASIC for nonlinear curve fitting. J. Pharmacol. Methods 14: 323–329.

KARLSSON-NORRGREN, L., W. DICKSON, O. LJUNGBERG, AND P. RUNN. 1986. Acid water and aluminum exposure: gill lesions and aluminum accumulation in farmed brown trout, *Salmo trutta* L. J. Fish Dis. 9: 1–9.

LAUREN, D. J., AND MCDONALD. 1987a. Acclimation to copper by rainbow trout, *Salmo gairdneri*: I. Physiology. Can. J. Fish. Aquat. Sci. 44: 99–104.

1987b. Acclimation to copper by rainbow trout, *Salmo gairdneri*: II. Biochemistry. Can. J. Fish. Aquat. Sci. 44: 105–111.

LITCHFIELD, J. T. 1949. A method for rapid graphic solution of time – percent effect curves. J. Pharmacol. Exp. Ther. 97: 399–408.

LITCHFIELD, J. T., AND F. WILCOXON. 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96: 99–113.

MALLATT, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can. J. Fish. Aquat. Sci. 42: 630–648.

MARSHALL, W. S. 1978. On the involvement of mucous secretion in teleost osmoregulation. Can. J. Zool. 56: 1088–1091.

MCDONALD, D. G. 1983. The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. I. Branchial and renal net ion and H^+ fluxes. J. Exp. Biol. 102: 123–140.

MCDONALD, D. G., AND C. L. MILLIGAN. 1988. Sodium transport in the brook trout, *Salvelinus fontinalus*: effects of prolonged low pH exposure in the presence and absence of aluminum. Can. J. Fish. Aquat. Sci. 45: 1606–1613.

MCDONALD, D. G., C. M. WOOD, R. G. RHEM, M. E. MUELLER, D. R. MOUNT, AND H. L. BERGMAN. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalus*). I. Physiology. Can. J. Fish. Aquat. Sci. 48: 2006–2015.

MCWILLIAMS, P. G. 1983. An investigation of the loss of bound calcium from the gills of the brown trout, *Salmo trutta*, in acid media. Comp. Biochem. Physiol. 74A: 107–116.

MILLIGAN, C. L., AND C. M. WOOD. 1982. Disturbances in hematology, fluid volume distribution, and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. J. Exp. Biol. 99: 397–415.

MUNIZ, I. P., AND H. LEIVESTAD. 1980. Toxic effect of aluminum on the brown trout, *Salmo trutta* L, p. 320–321. *In* Proceedings of the International Conference on the Ecological Impact of Acid Precipitation. S.N.S.F. project Oslo, Norway.

NEVILLE, C. M. 1985. Physiological response of juvenile rainbow trout, *Salmo gairdneri*, to acid and aluminum — prediction of field responses from laboratory data. Can. J. Fish. Aquat. Sci. 42: 2009–2019.

OGASAWARA, T., AND T. HIRANO. 1984. Changes in osmotic water permeability of the eel gills during seawater and freshwater adaptation. J. Comp. Physiol. 154: 3–11.

ORR, P. L., R. W. BRADLEY, J. B. SPRAGUE, AND N. J. HUTCHINSON. 1986. Acclimation-induced change in toxicity of aluminum to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 243–246.

PAGENKOPF, G. K. 1983. GIll surface interaction model for trace metal toxicity to fish: role of complexation, pH, and water hardness. Environ. Sci. Toxicol. 17: 347–352.

PART, P., AND R. A. C. LOCK. 1983. Diffusion of calcium, cadmium and mercury in a mucous solution from rainbow trout. Comp. Biochem. Physiol. 76C: 259–263.

REID, S. D. 1989. Gill–metal interactions in rainbow trout (*Oncorhynchus mykiss*). Ph.D. thesis, McMaster University, Hamilton, Ont. 172 p.

SANDOR, T., J. A. DIBATTISTA, AND A. Z. MEDHI. 1984. Glucocorticoid receptors in the gill tissue of fish. Gen. Comp. Endocrinol. 53: 353–364.

SCATCHARD, G. 1949. The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51: 660–672.

SCHOFIELD, C. L., AND J. R. TROJNAR. 1980. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters p. 341–363. *In* T. Y. Toribara, M. W. Miller, and P. E. Morrow [ed.] Polluted rain. Plenum Press, New York, NY.

VAN DE WINKEL, J. G., T. H. M. S. M. VAN KUPPEVELT, H. M. J. JANSSEN, AND R. A. C. LOCK. 1986. Glycosaminoglycans in the skin mucus of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 85B: 473–475.

WARREN, L. 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234: 1971–1975.

1963. Thiobarbituric acid assay of sialic acids, p. 463–465. *In* S. Colowick and N. O. Kaplan [ed.] Methods in enzymology. Academic Press, New York, NY.

WILLIAMS, R. J. P. 1979. Cation and proton interactions with proteins and membranes. Biochem. Soc. Trans. 7: 481–509.

WOLD, J. K., AND R. SELSET. 1977. Glycoproteins in the skin mucus of the char (*Salmo alpinus* L.). Comp. Biochem. Physiol. 56B: 215–218.

WOOD, C. M., R. C. PLAYLE, B. P. SIMONS, G. GOSS, AND D. G. MCDONALD. 1988b. Blood gasses, acid base, ions, and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure. Can. J. Fish. Aquat. Sci. 45: 1575–1586.

WOOD, C. M., D. G. MCDONALD, C. E. BOOTH, B. P. SIMONS, C. G. INGERSOL, AND H. L. BERGMAN. 1988a. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. 45: 1587–1596.

WOOD, C. M., B. P. SIMONS, D. R. MOUNT, AND H. L. BERGMAN. 1988c. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 2. Blood parameters by cannulation. Can. J. Fish. Aquat. Sci. 45: 1597–1605.

WRIGHT, R. F., AND E. T. GJESSING. 1976. Acid precipitation: changes in the chemical composition of lakes. Ambio 5: 219–223.

YOUSON, J. H., AND C. M. NEVILLE. 1987. Deposition of aluminum in the gill epithelium of rainbow trout (*Salmo gairdneri* Richardson) subjected to sublethal concentrations of the metal. Can. J. Zool. 65: 647–656.

ZUCHELKOWSKI, E. M., C. A. PINKSTAFF, AND D. E. HINTON. 1985. Mucosubstance histochemistry in control and acid-stressed epidermis of brown bullhead catfish, *Ictalurus nebulosus* (LeSueur). Anat. Rec. 212: 327–335.

Nature and Time Course of Acclimation to Aluminum in Juvenile Brook Trout (*Salvelinus fontinalis*). I. Physiology

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McDonald, D. G., C. M. Wood, R. G. Rhem, M. E. Mueller, D. R. Mount, and H. L. Bergman. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). I. Physiology. Can. J. Fish. Aquat. Sci. 48: 2006–2015.

Chronic exposure (up to 42 d) of juvenile brook trout (*Salvelinus fontinalis*) to sublethal aluminum at pH 5.2 resulted in a damage/repair acclimation phenomenon. The damage developed rapidly (within 24 h), was centered at the gills, and was characterized by substantial accumulation of Al, a corresponding reduction of gill sialic acid content (a measure of gill mucus), and inhibition of branchial Na^+ transport. The corresponding internal effects of this initial damage were losses of electrolytes, hemoconcentration, and impaired tissue O_2 delivery (as indicated by elevated lactate). Repair was characterized by progressive reduction of gill Al, restoration of sialic acid content, recovery of Na^+ transport, and reduction in hemoconcentration and lactate levels. Accompanying the recovery was progressive development (by day 10 onwards) of increased resistance (i.e. acclimation) to acutely lethal Al. This acclimation was characterized by a reduction in both the rate of mortality and in the magnitude of physiological disturbances relative to control (i.e. Al naive) fish. The increased short-term resistance translated to greatly improved survivorship and correspondingly diminished physiological impact in the face of chronically elevated Al levels (2 wk at <300 μg Al/L). The acclimation process clearly resulted from specific changes at the gills.

L'exposition chronique jusqu'à 42 jours d'ombles de fontaine juvéniles à des concentrations sublétales d'aluminium dans un milieu de pH 5,2 a donné lieu à un phénomène d'acclimation suivant un cycle perturbations/rétablissement. Les perturbations, qui se sont présentées en deçà de 24 h, touchaient surtout les branchies. Elles étaient caractérisées par une importante accumulation d'Al, une baisse correspondante de la teneur en acide sialique dans les branchies (une quantification du mucus des ouïes) et l'inhibition du transport du Na^+ dans les branchies. Les répercussions internes correspondantes de ces perturbations initiales concernaient la perte d'électrolytes, l'hémoconcentration et la réduction de l'apport d'O_2 dans les tissus (comme l'ont révélé les teneurs élevées en lactate). Le rétablissement était caractérisé par une diminution graduelle de la teneur en Al dans les branchies, le rétablissement de la teneur en acide sialique, le rétablissement du transport du Na^+ et une baisse de l'hémoconcentration et des teneurs en lactate. L'élaboration progressive à partir du dizième jour d'une résistance accrue (c'est-à-dire l'acclimatation) à des teneurs létales aiguës d'Al suivait le processus de rétablissement. Cette acclimatation était caractérisée par une baisse du taux de mortalité et de l'ampleur des perturbations physiologiques par rapport aux poissons témoins qui n'avaient jamais été exposés à l'Al. Cette résistance accrue à court terme s'est traduite par un taux de survie nettement amélioré et une incidence physiologique diminuée porportionnellement en présence de teneurs en Al élevées de façon chronique (2 sem à moins de 300 μg Al/L). Le processus d'acclimatation était clairement le résultat de modifications particulières survenues dans les branchies.

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An important correlate of environmental acidification is elevation in dissolved aluminum; levels in excess of 100 μg/L have been reported in lakes and rivers still supporting fish populations (Wright and Snekvik 1978; Schofield and Trojnar 1980; Kelso et al. 1986). This has prompted investigators to question the impact on fish of chronic sublethal Al exposure. Studies to date are generally agreed that prolonged (i.e. >1 wk to several weeks) exposure to sublethal Al conveys increased resistance to short-term increases in Al (Guthrie 1982; Orr et al. 1986; Siddens et al. 1986; Wood et al. 1988a, 1988b; McDonald and Milligan 1988), i.e. fish acclimate to Al. Furthermore, this is an effect specifically of Al rather than the low pH's which invariably accompany elevated Al (Wood et al. 1988a, 1988b). The Al acclimation takes the form of a reduced rate of mortality in the face of lethal Al levels (Guthrie 1982; Wood et al. 1988a, 1988b), an increase in the toxic threshold to Al (specifically, an increase in the 96-h, LC_{50}; Orr et al. 1986), and a reduction in the magnitude of physiological disturbances (Wood et al. 1988a, 1988b; McDonald and Milligan 1988).

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The physiological effects of Al (impaired ionoregulation and gas exchange) indicate that the gills are the main target organ. Thus, we hypothesize that the acclimation process arises from specific biochemical, physiological, and/or morphological changes in the gills. In our previous studies, we showed that Al acclimation led to an improvement of ionoregulatory function in the face of Al challenge; specifically, lower net Na^+ losses during elevated Al and a more rapid recovery of net Na^+ balance (Wood et al. 1988a, 1988b). The increased resistance of Na^+ regulation was, at least in part, due to changes in branchial active Na^+ transport; fish maintained at low pH (pH 5.2) in the presence of Al had a higher rate of branchial Na^+ uptake than fish maintained at pH 5.2 alone or those maintained at pH 6.5 (McDonald and Milligan 1988). These findings were obtained on brook trout (*Salvelinus fontinalis*) exposed 10 wk to Al; presumably the Al adaptation process was complete by this time.

Thus the objective for this and the companion study (Mueller et al. 1991) was to examine both the time course and the nature of acclimation to Al in brook trout. The focus of the present study was upon various measures which either directly or indirectly assess the physiological state of the gills (gill mucous and Al content, Na^+ transport activity, body electrolytes, lactate, and hematology) whereas Mueller et al. (1991) focus specifically on changes in gill morphology.

Methods

Experimental Animals and Holding Conditions

Experiments were conducted at the Fish Physiology and Toxicology Laboratory, Laramie, WY, which provided facilities for continuous exposure of fish to defined pH, Ca^{2+}, and Al conditions in flowing artificial soft water. Artificial soft water (composition in Table 1) was generated by treatment of hard well water ($Na^+ = 0.3$ mequiv/L, $Ca^{2+} = 2.6$ mequiv/L) with sediment filtration, NaCl softening, reverse osmosis, and separate bed deionization. The pH was adjusted to 6.5 with KOH and the water was thoroughly mixed and then delivered to individual head tanks where the pH was lowered to the desired level with H_2SO_4. The carbonate alkalinity of this water at pH 6.5 was about 2.5 mg/L. All pH adjustments were made with Leeds and Northrup pH controllers (pH analyzer/controller model No 7083). The required levels of $CaCl_2$ and $AlCl_3$ were added via Mariotte bottles to continuous-flow serial diluters. From here the water was delivered to the exposure tanks, and then to waste. The tanks were round, 800-L fiberglass chambers served with a flow of 4 L/min, providing 7 volume additions per day.

Test animals were juvenile brook trout (2–13 g) obtained from Cline's Trout Farm, Boulder, CO, and initially maintained with periodic feeding in hard well water. Prior to softwater acclimation, the fish were fin clipped for later separation into three groups and then transferred to soft water for an acclimation period of 14 d. Experimental temperature was 10 ± 1°C. Fish were fed floating feed (Purina Trout Chow) at 1% body weight/day and tanks were siphoned daily to remove organic debris. Photoperiod was adjusted biweekly to follow the natural cycle for Laramie. Water chemistry was measured daily on samples taken directly from the tanks.

Experimental Protocol

Brook trout were exposed to one of three acclimation conditions (Group I, Table 1): normal soft water (pH 6.5, Ca^{2+} = 25 μequiv/L, Al = 0; 6.5/0 group), low-pH soft water (pH 5.2, Ca^{2+} = 25 μequiv/L, Al = 0; 5.2/0 group), and low-pH soft water + Al (5.2/Al group) for up to 42 d. One tank was used for each exposure and each tank started with 400–600 fish. The initial Al level for the 5.2/Al group was 150 μg/L but this

TABLE 1. Nominal exposure conditions and measured water chemistry (means and ranges) during 24-d chronic exposures. Group I data are for exposures shown in Fig. 2. All levels were reduced from 150 to 75 μg/L on day 3 (see Fig. 3). Group II data are for exposures conducted 6 mo later (see Fig. 3).

Nominal			Measured					
pH	Ca^{2+} (μequiv/L)	Al (μg/L)	pH	Temperature (°C)	Ca^{2+} (μequiv/L)	Total Al (μg/L)	Na^+ (μequiv/L)	Cl^- (μequiv/L)
				Group I				
6.5	25	0	6.60 (6.35–6.82)	9.1 (9.0–9.6)	28 (23–32)	0.9 (<1–3.7)	43.9 (39.6–47.4)	43.4 (40.9–45.7)
5.2	25	0	5.13 (4.97–5.32)	9.2 (8.9–9.6)	26 (22–29)	1.4 (<1–3.7)	43.9 (40.0–47.9)	45.1 (40.9–57.8)
5.2	25	150 (0–3 d)	5.26 (5.20–5.37)	9.0 (9.0)	22 (22–23)	164.5 (157.4–171.6)	50.0 (49.2–50.9)	64.0 (61.5–66.3)
5.2	25	75 (3–24 d)	5.16 (5.01–5.23)	9.2 (9.0–9.5)	27 (23–29)	68.2 (55.6–78.3)	42.6 (39.1–44.4)	48.8 (48.2–50.2)
				Group II				
6.5	25	0	6.48 (6.02–6.79)	10.2 (10.0–10.5)	36 (33–38)	0.0	56.5 (49.2–61.3)	57.5 (46.3–70.0)
5.2	25	0	5.29 (5.08–5.61)	10.3 (10.0–10.5)	37 (32–40)	0.0	58.3 (46.1–67.4)	57.5 (48.0–66.6)
5.2	25	75	5.12 (51.0–5.37)	9.9 (9.5–10.1)	34 (29–38)	70.2 (65.0–75.2)	56.1 (45.7–63.9)	63.8 (51.3–73.3)
5.2	25	150	5.09 (4.91–5.39)	9.9 (9.5–10.0)	34 (28–38)	145.2 (140.8–148.0)	65.2 (47.4–75.7)	81.5 (61.5–92.2)

was reduced on day 3 to 75 μg/L to reduce mortalities and was maintained at 75μg/L thereafter (Table 1, Group I). To assess the effect of this first 3-d exposure to a variable Al regime, a second acclimation experiment (Group II, Table 1) was conducted 6 mo later. Brook trout (8–46 g) were obtained from the same source and acclimated to soft water for 14 d. They were then divided into four exposure groups: 6.5/0 ($N=60$), 5.2/0 ($N=60$), 5.2/75 ($N=100$), and 5.2/150 ($N=100$) and maintained under these conditions for up to 10 d. Fish ($N=10$ from each group) were sampled at periodic intervals (days 1, 2, 4, 7, and 10; e.g. see Fig. 3) and analyzed for whole-body electrolytes and gill Al.

To assess the nature and time course of acclimation in Group I fish, animals were periodically sampled from each acclimation condition for measurements of physiological state or for challenge with higher Al levels. Physiological measures included whole-body electrolytes (Na^+, Cl^-), whole-body lactate, hematology (hematocrit and plasma protein), gill chemistry (sialic acid and Al content), and branchial Na^+ fluxes (influx, J_{in}, and net flux, J_{net}). Resistance to challenge was assessed on the basis of cumulative mortality over time and by the magnitude of physiological disturbances in survivors at 24 h.

From day 0 to 24 of the acclimation experiment, 52 fish from each acclimation group were removed at each sampling time (days 1, 2, 4, 7, 10, 13, 17, and 24; eg. see Fig. 2). Sixteen of these fish were used for measurements of Na^+ uptake; half were subsequently returned to their respective acclimation tank and the other half killed for measurements of whole-body electrolytes. Another 10 fish were immediately killed and a blood sample obtained by caudal puncture using a modified 100-μL Hamilton gas-tight syringe. Hematocrit and plasma protein were determined on this blood sample. The second gill arch from the right side was taken for histological examination (see companion study by Mueller et al. 1991) and the remaining gill basket was weighed, frozen, and later digested for gill Al and sialic acid determination. A third group of 10 fish from each acclimation group was transferred to a single challenge tank (pH 5.2, nominal Al = 1000 μg/L) and cumulative mortalities recorded over time until all fish were dead. An estimate of the time to 50% mortality (LT_{50}) was determined by log-probit analysis for each group exposed to the challenge. A fourth group of 16 fish from each acclimation group was challenged simultaneously with the same condition; survivors at 24 h were used for either (i) determinations of whole body electrolytes and lactate or (ii) measurements of hematocrit, plasma protein, gill Al and sialic acid content, and gill histology.

A final challenge experiment was begun on day 28 of the acclimation experiment. Here, a total of 90 fish from each of the 6.5/0 and 5.2/Al groups were transferred in groups of 15 to one of six exposure tanks (pH 4.8 at 0, 100 or 300 μg Al/L; pH 5.2 at 0, 100 or 300 μg Al/L). Fish were maintained for 2 wk under these conditions and cumulative mortalities recorded over time. At the end of the 2 wk, up to 10 survivors from each group in each tank were then measured for whole-body electrolytes, hematology, and gill Al.

Analytical Techniques and Calculations

Whole-body lactate and electrolytes

After a killing blow to the head, fish were grasped immediately with aluminum tongs and freeze-clamped at −80°C in liquid N_2 (~5 s), weighed, and homogenized in 30 mL of ice-cold 8% perchloric acid in a food chopper (Black & Decker HC 20). The homogenate was allowed to stand at 4°C for 2 h and then centrifuged at 900*g* for 10 min. Duplicate 100-μL aliquots of the supernatant were analyzed enzymatically for lactate (L-lactate dehydrogenase/NADH method; Loomis 1961; Sigma reagents). Triplicate 100-μL aliquots of supernatant were measured for Na^+ by AAS (Perkin Elmer atomic absorption spectrophotometer, model 2380) following 1:100 dilution in deionized water. Triplicate 200- to 400-μL aliquots were measured for Cl^- directly by coulometric titration with a Radiometer CMT-10 chloridometer.

Blood measurements

Blood samples (50–200 μL) obtained by caudal puncture were centrifuged in heparinized microcapillary tubes at 5000*g* for 5 min. Percent red cell volume (hematocrit) was measured and plasma drawn off for immediate analysis of protein. Plasma protein was determined with an American Optical Goldberg refractometer (Alexander and Ingram 1980).

Gill aluminum and sialic acid content

Gills (whole branchial baskets minus the second arch on the right side) were digested in 0.1 N H_2SO_4 (5.5. mL acid/g gill tissue) at 80°C overnight. Triplicate aliquots (10–25 μL) of this digest were analyzed for Al with the pyrocatechol violet method (Dougan and Wilson 1974) using a standard curve prepared from a digest of gill tissues collected on day 0 from 6.5/0 animals, i.e. animals that had not been exposed to Al.

Duplicate 25-μL aliquots of the gill digest were analyzed for sialic acid content using the thiobarbituric method of Warren (1963). This assay is used as a convenient method for estimating the mucous content of the gills (e.g. Arillo et al. 1979). Sialic acids are the main acidic component of fish mucus (Harris et al. 1973). Standard curves were prepared using *N*-acetylneuramic acid (NANA, obtained from Sigma). According to Zuchelkowski et al. (1985), NANA is the most common form of sialic acid produced in fish mucous cells.

Na^+ uptake

Na^+ transport was assessed by examining the relationship between unidirectional Na^+ uptake (J_{in}^{Na+}) and water Na^+ concentration ([Na^+]) at pH 6.5. Fish were transferred from their respective acclimation tanks (16 from each group) to individual 250-mL chambers containing normal soft water (pH 6.5, Al = 0; Table 1). Each chamber was immersed in a 10°C water bath and was fitted with an air line which provided aeration and mixing. Each acclimation group was exposed to four Na^+ levels ($N=4$ at each level; 0.45, 0.75, 1.45, and 2.85 mequiv/L) and Na^+ fluxes measured for 1 h. Na^+ was added as NaCl to each chamber along with $^{22}Na^+$. The amount of $^{22}Na^+$ was varied so that specific activity remained constant at 0.9 μCi/mequiv (1 μCi = 37 kBq). Water samples (14 mL) were taken 5 min after Na^+ addition (to allow for mixing) and again at the end of 1 h. Water samples were analyzed in duplicate for [Na^+] by AAS and for $^{22}Na^+$ activity by scintillation counting (5 mL of water + 10 mL of ACS fluor) in a Beckman model LS9000 scintillation counter.

J_{in}^{Na+} (nanoequivalents per gram per hour) was determined according to the following formula:

$$J_{in}^{Na+} = \frac{(cpm_i - cpm_f) \times vol}{time \times wt \times SA}$$

where cpm is the concentration of radiotracer ($^{22}Na^+$) in the medium (counts per minute per millilitre) at the beginning (*i*)

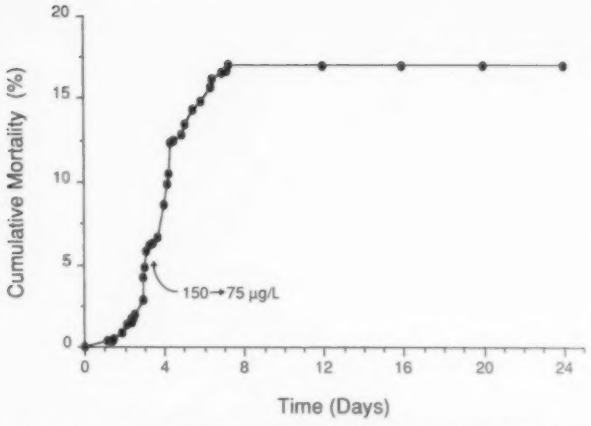

FIG. 1. Cumulative mortality during chronic Al exposure at pH 5.2 in juvenile brook trout at 10°C ($N = 600$ on day 0). On day 3, water Al levels were decreased from 150 to 75 µg/L (indicated by arrow).

and end (f) of the measurement period, vol is the volume (millilitres) of the flux chamber, SA is the radiospecific activity of the external medium (cpm per nanoequivalent), time is in hours, and wt is the animal's body weight (grams).

Net Na^+ flux (J_{net}^{Na+}, (nanoequivalents per gram per hour) was determined from changes in Na^+ concentration in the water:

$$J_{in}^{Na+} = \frac{([Na^+]_i - [Na^+]_f) \times vol}{time \times wt}.$$

Treatment of Data

Data have been reported as means ± 1 SE for each group (i.e. each exposure condition). One-way analysis of variance was used to assess differences among groups; when the F value indicated significance, Fisher's protected least significant difference (PLSD) was applied to discern specific differences (Winer 1971, p. 201). Estimates of LT_{50} were computed by log-probit analysis of time/percent effect data. Nomographic methods outlined in Litchfield (1949) and Litchfield and Wilcoxon (1949) were used to compute 95% confidence limits for the log-probit regressions. LT_{50}'s were considered significantly different when the confidence limits did not overlap. A 5% significance level was used throughout.

Results

"Sublethal" Al Exposure

An Al level of 150 µg/L (nominal, see Table 1) was chosen for the Al acclimation study, since we had previously shown (Wood et al. 1988a) that this level was sublethal to adult brook trout but would still evoke the acclimatory response. Furthermore, initial 48-h range-finder tests confirmed sublethality to juveniles. However, once the acclimation began, initial mortalities were unacceptably high; they had reached 4% by day 3 and were continuing (Fig. 1). Consequently, there was a risk of biasing this experiment by selection for hardier individuals rather than examining acclimation per se. Accordingly, the Al level was reduced from 150 to 75 µ/L on day 3 (indicated by arrow in Fig. 1). Although mortalities continued to occur, they eventually leveled out at 17% by day 7 of the exposure. In contrast, no mortalities occurred in the two other exposures, 5.2/0 and 6.5/0.

The control or 6.5/0 fish, while showing no consistent change in any measured parameter over time, did show a daily variation of up to 10% from overall means. Mean values for each measured parameter computed for the 24-d control period (1 ± SEM, $N = 80$) are shown on the broken lines in Fig. 2. To facilitate the comparison between the control and treatment groups (5.2/0 and 5.2/Al), and to correct for the daily variation, all measured variables on the treatment groups have been expressed as a difference from control fish *measured on the same day*.

Fish exposed to Al (150 → 75 µg/L) at pH 5.2 showed significant physiological disturbances relative to controls (Fig. 2): a depression in whole-body Na^+ and Cl^- of nearly equal magnitude (Fig. 2A and 2B); an increased hematocrit and plasma protein concentration (Fig. 2C and 2D), due likely to transcellular fluid shifts and hemoconcentration resulting from the electrolyte losses; and a significant elevation in whole-body lactate (Fig. 2E), probably resulting from impaired O_2 delivery to tissues. These physiological disturbances developed quickly, reached a peak at about 4 d of Al exposure, and thereafter gradually abated. Body Na^+ and Cl^- levels stabilized at about 80% of controls by 24 d of exposure but were still significantly depressed. Similarly, there was still a significant hemoconcentration evident. In contrast, body lactate levels had returned to control levels by day 17 of Al exposure.

Over the first 24 h of Al exposure, gill Al levels rose sharply, reaching a peak of 251 ± 30 µg/g wet gill tissue, and thereafter declining to a steady-state level of about 40 µg/g by day 10 (Fig. 2F). Correlated with the high gill Al levels was a significant depression in gill sialic acid content. Over the first week of the exposure, sialic acid levels were consistently about 35% less than controls: 1.68 ± 0.13 ($N = 38$) versus 2.55 ± 0.19 ($N = 40$) µmol/g wet gill tissue in the 5.2/Al and 6.5/0 groups, respectively. After 1 wk the sialic acid content of the gills of the 5.2/Al group increased to control levels, and thereafter the two groups were not significantly different from one another.

The fish exposed to pH 5.2 alone also showed some significant disturbances, indicating that they too were stressed. These consisted of a depression in body electrolytes late in the exposure period (Fig. 2A and 2B), some elevation of plasma protein (Fig. 2D) but not of hematocrit (Fig. 2C), and a significant elevation of body lactate, but only on one day (day 12; Fig. 2E). Not surprisingly, gill Al was no different than controls in this group, and was not significantly different than zero. Gill sialic acid content also remained virtually identical to controls at a mean level of 2.65 ± 0.12 ($N = 67$) µmol/g throughout the 24-d exposure.

The variable Al regime over the first few days of this experiment prompted the second series of exposures (Group II, Table 1) where the effects of the two Al levels (75 and 150 µg/L) were compared at pH 5.2 (Fig. 3). The main finding of this experiment was that exposure to constant 75 µg/L produced the same disturbance to body electrolytes as the exposure to pH 5.2 alone (Fig. 3A and 3B), i.e. this suggests that Al = 75 µg/L was below the threshold required to produce an Al-specific effect. In contrast, the constant 150 µg/L exposure produced the same disturbance as the 150 → 75 exposure (Fig. 3A and 3B). This suggests that with above-threshold Al levels, the critical period is the initial stages of the exposure. Accompanying the 150 → 75 and 150 exposures was substantial

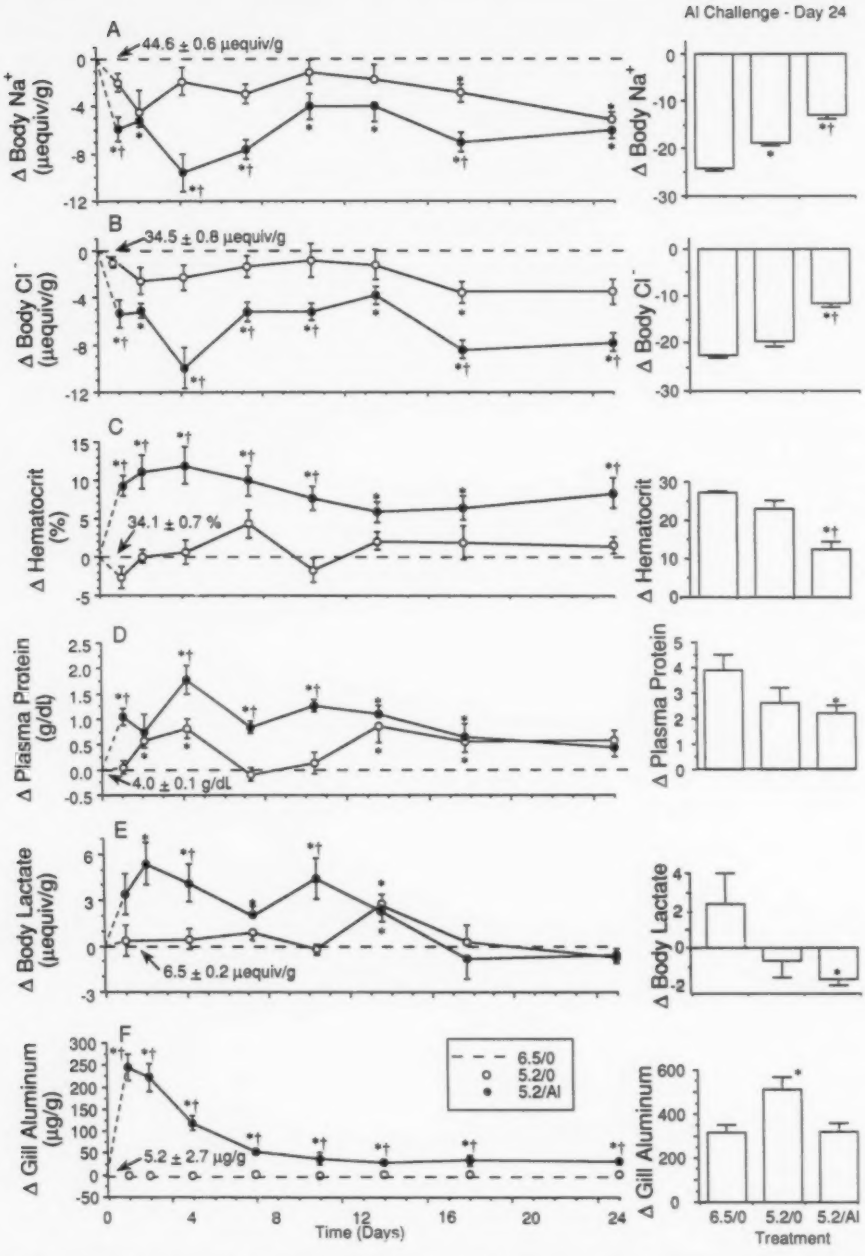

FIG. 2. (A) Whole-body Na^+, (B) whole-body Cl^-, (C) hematocrit, (D) plasma protein, (E) whole-body lactate, and (F) gill Al during 24 d of chronic exposure to Al (150 → 75 µg/L, left-hand panel) and following 24 h of acutely lethal Al challenge started on day 24 (right-hand panel; challenge = pH 5.2, 746 µg Al/L). Values on the broken lines are the averages for the whole 24-d period for the 6.5/0 (control) group (means ± 1 SE $N = 80$). Daily means (± SE) for 5.2/0 fish (open circles) and 5.2/Al fish (closed circles) are expressed as the difference from the mean for control fish measured on the same day. In the Al challenge (right-hand panel), values indicated are the differences (mean ± 1 SE) from the chronic exposure measurements on the same group made 24 h earlier. Asterisks indicate means (± 1 SE, $N = 8$–10) significantly different ($p < 0.05$) from 6.5/0; crosses indicate means significantly different from 5.2/0.

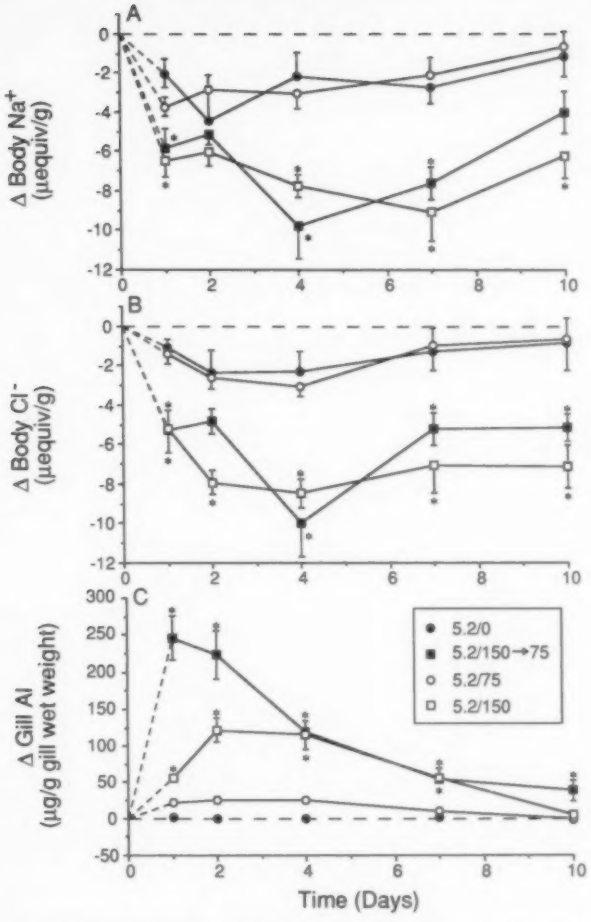

FIG. 3. (A) Whole-body Na^+, (B) whole-body Cl^-, and (C) gill Al during 10 d of acclimation to pH 5.2 and four different Al regimes (0, 75, 150 → 75, and 150 μg/L). Closed symbols are data from the first chronic exposure experiment taken from Fig. 2; open symbols are data from the second chronic exposure experiment conducted 6 mo later (Group II, Table 1). All values are mean differences from controls (6.5/0) measured on same day (± 1 SE, $N = 8$–10). Asterisks indicate means significantly different ($p < 0.05$) from corresponding means in the experiments conducted at the same time (i.e. ● versus ■, ○ versus □).

initial accumulation of Al on the gills (Fig. 3C). In contrast, there was only trace accumulation in the fish at constant 75 μg/L, further reinforcing the notion that this level of Al was below threshold (Fig. 3C). For some unknown reason, the initial gill in the 150 → 75 group was greater than in the constant 150 group. Nonetheless, by day 4, both groups had virtually identical Al levels, and thereafter, gill levels declined more or less in parallel in both groups, despite the continued presence of Al in the water.

Na^+ Transport Activity

In control (6.5/0) fish measured at pH 6.5, there was a linear relationship between J_{in}^{Na+} and external [Na^+] over the tested range of 0.4–2.8 mequiv Na^+/L for each of the eight separate occasions over the 24 d that measurements were made (Fig. 4).

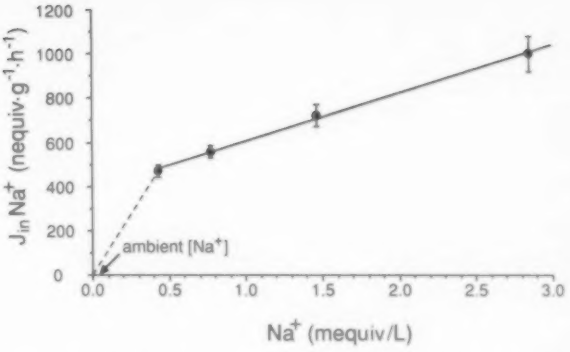

FIG. 4. Relationship between J_{in}^{Na+} and external [Na^+] in juvenile brook trout at pH 6.5. Each value is the mean (± 1 SE, $N = 32$) of measurements made on eight separate occasions during the 24-d chronic exposure experiment on groups of four different fish on each occasion. Line fitted by least squares regression; $J_{in} = 219 \cdot Na^+ + 387$, $r = 0.998$. Arrow indicates the [Na^+] level to which all fish were acclimated (0.04 mequiv/L).

In previous studies on brook trout (McDonald and Milligan 1988), we found a similar linear relationship. This linearity means that Na^+ transport activity cannot be expressed in the more conventional terms of saturation kinetics (i.e. maximum transport rate (J_{max}) and transport carrier affinity (K_m)). However, it remains possible that a saturable component exists in the untested range of 0.0–0.4 mequiv Na^+/L which encompasses ambient [Na^+] for these fish (0.04 mequiv/L; Table 1).

In the 5.2/0 and 5.2/Al groups, there were also linear relationships between J_{in}^{Na+} and [Na^+] although with different elevations. Thus, for the sake of simplicity, only the J_{in}^{Na+} measurements at the lowest [Na^+] (0.4 mequiv/L) have been plotted (Fig. 5A). The effect of Al exposure was an immediate and significant inhibition of Na^+ uptake (to about 50% of controls) by 24 h of exposure which gradually but almost completely recovered over the subsequent 23 d. Because the measurements of J_{in}^{Na+} in Al-exposed fish were made at pH 6.5 *without Al in the water*, this initial inhibition probably resulted from accumulated physiological damage to the gills. Similarly, the gradual recovery can be taken as evidence of repair of that damage.

No similar damage appears to have resulted from the pH 5.2 exposure. Over the first 10 d of pH 5.2 exposure, J_{in}^{Na+} was virtually identical to controls (Fig. 5A); thereafter, it was significantly higher than controls. The latter corresponds to the period where the 5.2/0 animals were showing significant depression of body Na^+ (Fig. 2A). This suggests that net Na^+ losses resulting from pH 5.2 exposure specifically stimulated the Na^+ uptake mechanism when the animals were briefly exposed to pH 6.5

Examination of J_{net}^{Na+} in each group ($N = 16$ at each measurement period; Fig. 5B) reveals the same basic trends as seen in the J_{in}^{Na+} measurements at the lowest Na^+ ($N = 4$; Fig. 5A). For the 6.5/0 group, there was very little variation with time in J_{net}^{Na+}, and overall, these fish exhibited a slight net uptake of Na^+, averaging 103 ± 76 nequiv·g^{-1}·h^{-1}, i.e. the animals were essentially in Na^+ balance. The other two groups showed significantly different responses over the first 10 d of exposure. The 5.2/0 group showed a greater net uptake relative to controls (Fig. 5B), while the 5.2/Al group showed a significant loss of Na^+ relative to controls (Fig. 5B), although they had lost the

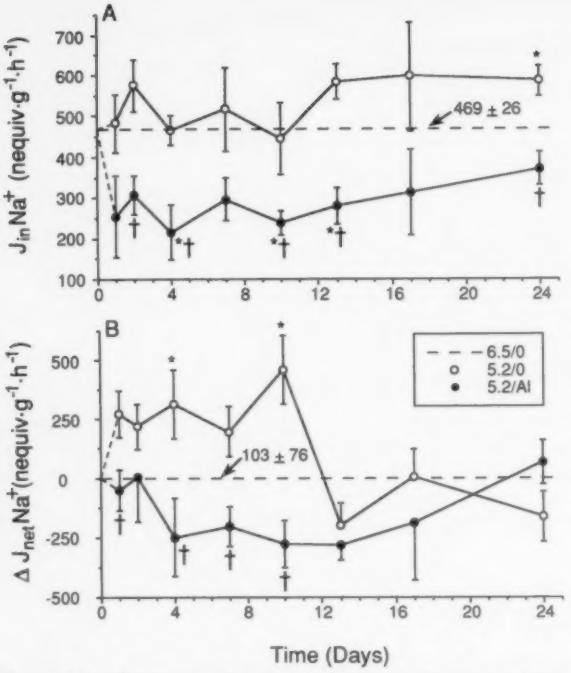

FIG. 5. (A) J_{in}^{Na+} measured at Na^+ = 0.4 mequiv/L (means ± 1 SE, $N=4$); (B) average J_{in}^{Na+} relative to controls (broken line) measured at Na^+ = 0.4–2.8 mequiv/L (means ± 1 SE, $N=16$). Asterisks indicate means significantly different ($p < 0.05$) from 6.5/0; crosses indicate means significantly different from 5.2/0.

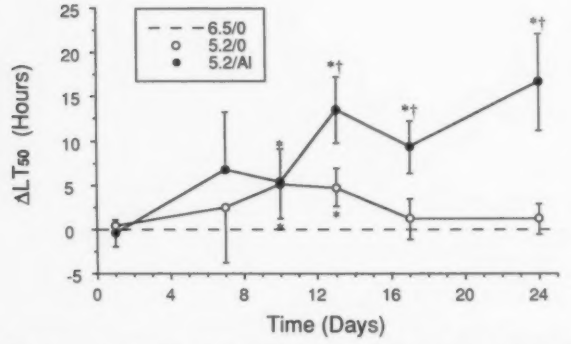

FIG. 6. Estimated LT_{50}'s in brook trout exposed to acutely lethal Al (607–1020 μg Al/L at pH 5.2). Open symbols indicate 5.2/0 fish and closed symbols indicate 5.2/Al fish, expressed as a difference (mean ± 95% confidence limits) from 6.5/0 fish measured on the same day. Asterisks indicate means significantly different ($p<0.05$) from 6.5/0; crosses indicate means significantly different from 5.2/0.

most Na^+ of the three groups (Fig. 2A and 2B). Clearly, the 5.2/0 group had retained the ability to restore electrolyte losses upon pH 6.5 exposure whereas that ability was significantly impaired in the 5.2/Al group.

Al Challenge during Acclimation

The Al challenge conditions were nominally 1000 μg Al/L at a pH of 5.2 for 48 h but, in fact, the actual mean Al value

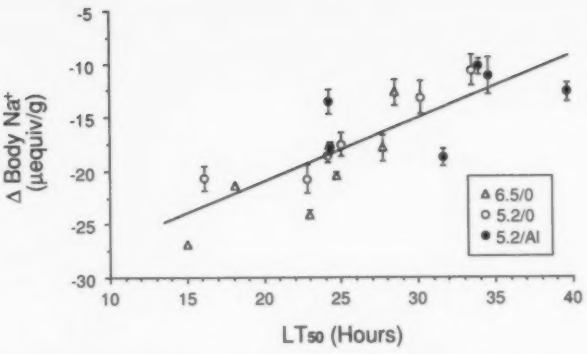

FIG. 7. Relationship between LT_{50} and loss of body Na^+ over the first 24 h of exposure in 24-h survivors for all three groups of fish. Data taken from all six challenge experiments (see Fig. 6). Line fitted by least squares regression; $\Delta NA^+ = 0.63 \cdot LT_{50} - 33.5$, $r = 0.835$.

for individual challenges varied from 610 to 1020 μg/L. Consequently, there was a corresponding variation in the LT_{50}'s, ranging, for example, in controls from 15 ± 1.3 to 33.8 ± 2.2 h. Since all three groups were exposed to Al in the same tank and thus to the same challenge conditions, there were similar variations in LT_{50} in the other two groups. Therefore, in order to clarify the differences amongst the groups, the LT_{50}'s for the 5.2/0 and 5.2/Al groups have been expressed relative to the controls (Fig. 6).

After 24 h of Al acclimation, the 5.2/Al group responded identically to challenge as controls, but from day 10 onward showed progressively greater Al resistance until day 24, where their LT_{50} was about 1.7-fold greater than controls. This was an effect primarily of Al, as the LT_{50}'s of the 5.2/0 group were virtually identical to controls throughout the acclimation period except for slightly higher values at days 10 and 12.

Not surprisingly, the Al challenge had a severe impact on physiological state as assessed from animals alive at 24 h. The degree of disturbance evident at 24 h was directly correlated with LT_{50}. This was evident in all three acclimation groups, as illustrated by the relationship between LT_{50} and Na^+ losses at 24 h (Fig. 7). This relationship emphasizes that electrolyte losses were the main cause of death by Al challenge, not only for the 6.5/0 group but for the 5.2/0 and the 5.2/Al groups as well.

A detailed view of the physiological effects of challenge is shown in Fig. 2 (right-hand bars); this is the challenge experiment started on day 24 of the acclimation ($LT_{50} = 23 \pm 1.5$ h for 6.5/0 fish). By 24 h of this challenge, fish from the 6.5/0 group had lost about 50% of their body electrolytes (Fig. 2A and 2B), leading to a pronounced hemoconcentration (doubling of both hematocrit and plasma protein; Fig. 2C and 2D) impaired O_2 delivery (50% increase in body lactate; Fig. 2E). These disturbances were accompanied by a substantial accumulation of gill Al (Fig. 2F). In this challenge, gill Al was 317 ± 34 μg/g wet gill tissue ($N=4$), but this was not a typical value for the 6.5/0 group. The typical value was, in fact, much higher, averaging 798 ± 62 μg/g overall ($N=34$). The accumulation of Al on the gills was similarly correlated with a significant depression in sialic acid content, as noted earlier during Al acclimation, but the depression was more substantial, to about 36%, on average, of control levels (0.98 ± 0.13 ($N=34$) versus 2.73 ± 0.15 μmol/g ($N=65$)).

The increased Al tolerance of the 5.2/Al fish by 24 d of chronic Al exposure was evident in significantly lower electrolyte losses resulting from the Al challenge compared with controls and correspondingly smaller hematological and lactate disturbances (Fig. 2A–2E). These fish, nonetheless, accumulated a virtually identical amount of Al on their gills (Fig. 2F) and there was a similar depression in gill sialic content.

Admittedly, the less severe loss of electrolytes in the 5.2/Al fish could be at least partly attributed to the fact that they started off the challenge with less to lose (Fig. 2A and 2B). Similarly, the 5.2/0 group started with less Na^+ and lost less relative to controls, yet showed no greater Al resistance after 24 d of exposure. However, it should be emphasized that in all three groups the physiological measurements were made on survivors taken at 24 h into the challenge experiment. About 50% of the animals in the 6.5/0 and 5.2/0 groups had already died prior to this sampling, while none had died in the 5.2/Al group. Consequently, the 6.5/0 and 5.2/0 groups would have been heavily selected for resistant individuals, while no such selection had occurred for the 5.2/Al fish. Thus, the effects of the Al challenge on the 6.5/0 and 5.2/0 population as a whole were likely much more severe than those indicated by the measurements in Fig. 2 whereas the 5.2/Al measurements were more representative.

Final Al Challenge

Although chronic sublethal Al exposure increased resistance to lethal Al over the short term, the question that remained was whether the 5.2/Al fish had acquired any long-term tolerance of Al. This was assessed in the final challenge experiment which began on day 28 by exposing fish from the 5.2/Al and 6.5/0 groups to various pH/Al combinations for a period of 14 d.

Mortality was dramatically less in the group preexposed to Al (Fig. 8A). Indeed, only 2 of 90 5.2/Al fish died over the 2-wk period compared with 34 of 90 6.5/0 fish. Mortality was correlated with Al in the latter and Al was more toxic at pH 5.2 than at pH 4.8. The biggest differences between the two groups were seen at the highest Al (300 μg/L) which caused only 2 mortalities out of 30 in the 5.2/Al group versus 28 out of 30 in the 6.5/0 group.

Correspondingly, there was a much greater physiological impact of Al on the 6.5/0 group. Net Na^+ losses (Fig. 8B) were directly correlated with mortality, and the increases in hematocrit were, in turn, correlated with the depression in body Na^+ (Fig. 8C). Accumulation of Al on the gills was generally greater at pH 5.2 than at pH 4.8 in relation to the greater toxicity of the former (Fig. 8D).

The 5.2/Al group, in contrast, actually showed some recovery in body Na^+ and hematocrit after 2 wk at pH 5.2 and 0 Al. This recovery was not complete, but rather was to levels identical to those of the 6.5/0 group exposed to 5.2/0. Nonetheless, their responses to elevated Al and lowered pH were considerably attenuated compared with the 6.5/0 group. The attenuation was most apparent at 4.8/300 where the loss in Na^+, the increase in hematocrit, and the increase in gill Al were less than half those seen in the 6.5/0 group. Again, it should be noted that the actual differences were probably greater, given the sampling bias discussed above. In this exposure, mortalities for the 5.2/Al and 6.5/0 groups were 7 and 87%, respectively; the measurements for the 6.5/0 group were based on two hardy survivors only.

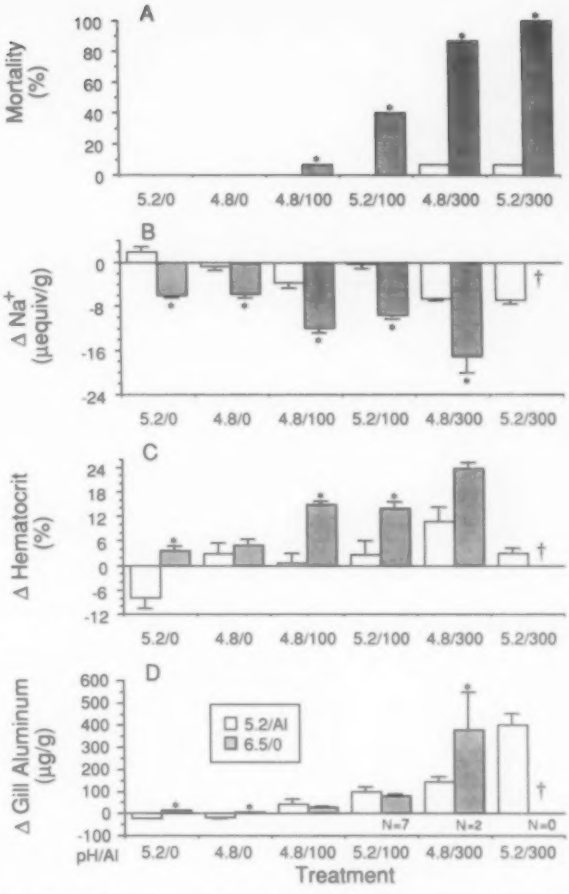

FIG. 8. (A) Percent mortality, (B) body Na^+, (C) hematocrit, and (D) gill Al in brook trout exposed 2 wk to low pH/Al (nominal values indicated). This is the final challenge experiment started on day 28 with 15 fish in each chronic exposure group. Open bars are 5.2/Al fish; shaded bars are 6.5/0 fish. Bars in Fig. 8B–8D indicate mean difference from the value in the same group prior to challenge (± 1 SE, $N = 10$ except where noted). Asterisks indicate significantly different ($p < 0.05$) means from 5.2/Al; Crosses indicates no survivors remaining after the 2-wk exposure.

Discussion

This study confirms and extends previous studies (Wood et al. 1988a, 1988b; McDonald and Milligan 1988) in finding that brook trout acclimation to Al. After only 10 d of exposure to largely sublethal Al, juveniles showed significantly increased resistance to lethal Al consisting of an increased LT_{50} and reduced physiological responses. The nature of the responses suggests that the acclimation most likely arose from changes at the gills. Questions that are thus important to answer concern the specific mechanism of action of Al at the gills and the precise nature of the adaptive changes that are brought about by sublethal Al exposure.

Mechanism of Toxic Action of Al

In previous brook trout studies (Booth et al. 1988), we established that in the initial (i.e. "shock") phase of Al exposure,

net ion losses arose primarily from large increases in diffusive ion fluxes across the gills, attributed to Al-induced increases in gill ionic permeability. Later on (in the "recovery" phase), as diffusive ion losses declined, the persistent inhibition of Na^+ and Cl^- uptake made a progressively greater contribution to the continuation of net ion losses or, in the present context, to the failure to completely recover preexposure electrolyte levels (Fig. 2A and 2B).

Clearly, the initial shock phase was very important in the present study, for it was during the first 24–48 h of the exposure to 150 μg Al/L that most of the physiological disruption (Fig. 2 and 3) and morphological damage (Mueller et al. 1991) took place. Earlier, Wood and McDonald (1987) and Booth et al. (1988) proposed that this initial phase was characterized by Al binding and/or precipitation at gill surfaces, specifically to anionic residues in mucous and outerfacing membranes. This action presumably displaced bound Ca^{2+}, weakened tight junctions, increased ionic permeability, and deformed and lifted the lamellar epithelium, causing increased exposure of binding sites, further gill Al accumulation, and further gill damage. In the present study, as shown previously (Booth et al. 1988), the gill Al accumulation was dramatic; 251 μg/g by 24 h of Al exposure (Fig. 2F) which represents a 1700-fold concentration over Al in the water (0.15 μg/mL). Correlated with the accumulation of Al was a 35% decline in gill sialic acid content. This is most readily interpreted as indicating a decline in gill mucous content, for in Salmonidae, sialic acid is the major cationic component of epithelial mucous (Harris et al. 1973) and its concentration is directly proportional to mucous cell density (Pickering 1974).

A decline in mucous content is unexpected given that hypertrophy and hyperplasia of gill mucous cells is often a correlate of Al exposure (e.g. Muniz and Leivestad 1980; McCahon et al. 1987) and, indeed, is a common response to gill irritants in general (Mallatt 1985). Nonetheless, if Al stimulated mucous secretion and sloughing to the extent that it exceeded mucous production, then sialic acid content would decline even in the face of mucous cell proliferation. Another possibility is that sialic acid content also declined out of proportion to mucous content. Zuchelkowski et al. (1985) reported increased synthesis of neutral sulfomucins (which do not contain sialic acid) in response to acid stress in catfish (*Ictalurus nebulosus*).

Whatever the changes in mucus, the gills did not continue to accumulate Al, nor did they remain leaky. By day 4, net ion losses appeared to have ceased and thereafter must have been reversed, as some recovery of Na^+ and Cl^- balance was evident (Fig. 2A and 2B). This recovery was accompanied by the removal of Al from the gills (Fig. 2F), restoration of sialic acid content, and almost complete recovery of branchial Na^+ transport (Fig. 5) and removal of the respiratory effect, as evidenced by the recovery of whole-body lactate levels (Fig. 2E). This was not simply restoration of the preexposure state, however, as development of resistance to Al was coincident with this recovery.

Mechanism of Acclimation to Al

So the question is "What specific changes have occurred in these gills so that they now better resist the toxic effects of Al?" The removal of Al from the gills during continued sublethal exposure (Fig. 2F and 3C) is perhaps the most important observation of the present study and may provide at least a partial answer to this question. The gills became less attractive to Al. Reid et al. (1991) have made a qualitatively similar observation on rainbow trout (*Oncorhynchus mykiss*) exposed to sublethal Al (27 μg/L) at the same pH (5.2). These findings argue for either an increased turnover rate of mucus at the gills, reducing the amount of Al held on the surface at any one time, or a decrease in the actual Al-binding affinity of the gill surface. Indeed, both mechanisms are likely. In rainbow trout, the extraction of Al from the water passing over the gills far exceeds the net accumulation of Al on the gills under these conditions, suggesting that the majority is sloughed off with mucus (Playle and Wood 1989). The companion histological examination (Mueller et al. 1991) of gills from brook trout in the present study demonstrates that gross hypertrophy and proliferation of branchial mucous cells coincided with the development of acclimation. Furthermore, Al-acclimated brook trout showed negligible hyperventilation during challenge, in contrast with naive fish, so less Al would be brought into contact with the gills (Walker et al. 1991). The in vitro studies of Reid et al. (1991) showed that quantitative and qualititative changes occurred in the cation-binding activity of the gills of rainbow trout during acclimation to Al. Specifically, these consisted of an increased binding affinity for Ca^{2+}, a decreased binding affinity for Al, and a decline in the potency of Al at displacing Ca^{2+}. These changes effectively reduced the surface activity of Al and led to about a 50% reduction of gill Al accumulation during Al challenge.

Unfortunately, the evidence that Al-acclimated brook trout actually accumulated less Al on the gills during challenge is, at best, marginal (Fig. 2F and 8D), in contrast with the clear results of Reid et al. (1991) on rainbow trout. While we cannot eliminate a true difference in mechanism between the two species, it seems more probable that the sampling bias during challenge, discussed earlier, prevented clear demonstration of the expected lower gill accumulation in acclimated brook trout. The actual propensity of the gills for Al is significantly lower in brook trout than in rainbow trout. For example, when both species were exposed to the same condition (48 h at pH 4.8, 111 μg Al/L), gill Al levels in the brook trout were only half those in the rainbow trout (D. G. McDonald and C. M. Wood, unpubl. results). This lower Al-binding activity in the brook trout may, in fact, underlie the intrinsically greater Al tolerance of this species.

In the Al-acclimated brook trout, the gills accumulated apparently similar amounts of Al as in unacclimated fish, but Al was clearly less reactive. Unfortunately, our physiological observations do not reveal the specific nature of these adaptive changes at the gills. They suggest repair of the initial damage rather than adaptation. Furthermore, the repair was not even complete over the period when acclimation was clearly established (days 10–24; Fig. 6), as evidenced by only partial correction and continued disturbance of Na^+ transport (Fig. 5), electrolyte levels, lactate levels, hematology (Fig. 2), and gill morphology (Mueller et al. 1991). Nonetheless, whatever the changes in the gills, the final challenge (Fig. 8) suggests that they convey relatively long-term tolerance of elevated Al. Consequently, in a field situation, fish chronically exposed to sublethal levels of Al might be able to survive otherwise lethal levels of Al during an episodic surge lasting perhaps as long as 2 wk.

Thus, we conclude that the adaptive response of the gills involves subtle but profound changes in the surface chemistry of the gills which may involve changes in rates of synthesis and/or turnover of membrane proteins, other proteins or lipids,

and/or de novo synthesis of new versions of these ligands with altered properties. Finally, we are left to ponder the metabolic costs and compromises associated with such a process, whether the adaptive changes convey resistance to any other toxicants, and how long the adaptive changes persist when Al is removed from the water. Questions such as these are, of course, not only applicable to Al but generally to chronic exposure of fish to sublethal toxicants.

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References

ALEXANDER, J. B., AND G. A. INGRAM. 1980. A comparison of five of the methods commonly used to measure protein concentrations in fish sera. J. Fish Biol. 16: 115–122.

ARILLO, A., C. MARGIOCCO, AND F. MELODIA. 1979. The gill sialic acid content as an index of environmental stress in rainbow trout, *Salmo gairdneri*, Richardson. J. Fish Biol. 15: 405–410.

BOOTH, C. E., D. G. MCDONALD, B. P. SIMONS, AND C. M. WOOD. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1563–1574.

DOUGAN, W. K., AND A. L. WILSON. 1974. The absorptiometric determination of aluminum in water. A comparison of some chromogenic reagents and the development of an improved method. Analyst 99: 413–430.

GUTHRIE, C. A. 1982. Acclimation of brook trout (*Salvelinus fontinalis*) to acidified waters. M.Sc. thesis, Cornell University, Ithaca, NY. 134 p.

HARRIS, J. E., A. WATSON, AND S. HUNT. 1973. Histochemical analysis of mucous cells in the epidermis of brown trout, *Salmo trutta* L. J. Fish Biol. 5: 345–351.

KELSO, J. R. M., C. K. MINNS, J. E. GRAY, AND M. L. JONES. 1986. Acidification of surface waters in eastern Canada and its relationship to aquatic biota. Can. Spec. Publ. Fish. Aquat. Sci. 87: 42 p.

LITCHFIELD, J. T. 1949. A method for rapid graphic solution of time – per cent effect curves. J. Pharmacol. Exp. Ther. 97: 399–408.

LITCHFIELD, J. T., AND F. WILCOXON. 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96: 99–113.

LOOMIS, M. E. 1961. An enzymatic fluorimetric method for the determination of lactic acid in serum. J. Lab. Clin. Med. 57: 966–972.

MALLATT, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can. J. Fish. Aquat. Sci. 42: 630–648.

MCCAHON, C. P., D. PASOCE, AND C. MCKAVANAGH. 1987. Histochemical observations on the salmonids *Salmo salar* L. and *Salmo trutta* L. and the ephemeropterans *Baetis rhodani* (Pict.) and *Ecdyonurus venosus* (Fabr.) following a simulated episode of acidity in an upland stream. Hydrobiologia 153: 3–12.

MCDONALD, D. G., AND C. L. MILLIGAN. 1988. Sodium transport in the brook trout, *Salvelinus fontinalis*: effects of prolonged low pH exposure in the presence and absence of aluminium. Can. J. Fish. Aquat. Sci. 45: 1606–1613.

MUELLER, M. E., D. A. SANCHEZ, H. L. BERGMAN, D. G. MCDONALD, R. G. RHEM, AND C. M. WOOD. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. Can. J. Fish. Aquat. Sci. 48: 2016–2027.

MUNIZ, I. P., AND H. LEIVESTAD. 1980. Acidification — effects on freshwater fish. Proc. Int. Conf. Ecol. impact acid Precip. SNSF project 84-92, Norway.

ORR, P. L., R. W. BRADLEY, J. B. SPRAGUE, AND N. J. HUTCHINSON. 1986. Acclimation-induced change in toxicity of aluminum to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 243–246.

PICKERING, A. D. 1974. The distribution of mucous cells in the epidermis of the brown trout, *Salmo trutta*, and the char, *Salvelinus alpinus*. J. Fish Biol. 6: 111–118.

PLAYLE, R. C., AND C. M. WOOD. 1989. Water pH and aluminum chemistry in the gill micro-environment of rainbow trout during acid and aluminum exposure. J. Comp. Physiol. B. 159: 539–550.

REID, S. D., D. G. MCDONALD, AND R. R. RHEM. 1991. Acclimation to sublethal aluminum: modifications of metal – gill surface interactions of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1996–2005.

SCHOFIELD, C. L., AND J. R. TROJNAR. 1980. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–363. *In* T. Y. Toribara, M. W. Miller, and P. E. Morrow [ed.] Polluted rain. Plenum Press, New York, NY.

SIDDENS, L. K., W. K. SEIM, L. R. CURTIS, AND G. A. CHAPMAN. 1986. Comparison of continuous and episodic exposure to acidic, aluminum-contaminated waters of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 43: 2036–2040.

WALKER, R. L., C. M. WOOD, AND H. L. BERGMAN. 1991. Effects of long-term preexposure to sublethal concentrations of acid and aluminum on the ventilatory response to aluminum challenge in brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 48: 1989–1995.

WARREN, L. 1963. Thiobarbituric acid assay of sialic acids, p. 463–465. *In* S. Colowick and N. O. Kaplan [ed.] Enzymatic methods. Academic Press, New York, NY.

WINER, B. J. 1971. Statistical principles in experimental design. 2nd ed. McGraw-Hill, New York, NY. 907 p.

WOOD, C. M., AND D. G. MCDONALD. 1987. The physiology of acid-aluminum stress in trout. Ann. Soc. R. zool. belg. 117: 399–410.

WOOD, C. M., D. G. MCDONALD, C. E. BOOTH, B. P. SIMONS, C. G. INGERSOLL, AND H. L. BERGMAN. 1988a. Physiological evidence of acclimation to acid-aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. 45: 1587–1596.

WOOD, C. M., B. P. SIMONS, D. R. MOUNT, AND H. L. BERGMAN. 1988b. Physiological evidence of acclimation to acid-aluminum stress in adult brook trout (*Salvelinus fontinalis*). 2. Blood parameters by cannulation. Can. J. Fish. Aquat. Sci. 45: 1597–1605.

WRIGHT, R. F., AND E. SNEKVIK. 1978. Chemistry and fish populations in 700 lakes in southernmost Norway. Verh. Int. Ver. Limnol. 20: 765–775.

ZUCHELKOWSKI, E. M., C. A. PINKSTAFF, AND D. E. HINTON. 1985. Mucosubstance histochemistry in control and acid-stressed epidermis of brown bullhead catfish, *Ictalurus nebulosus*. Anat. Rec. 212: 327–335.

Nature and Time Course of Acclimation to Aluminum in Juvenile Brook Trout (*Salvelinus fontinalis*). II. Gill Histology

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Gill samples from juvenile brook trout (*Salvelinus fontinalis*) acclimated to low-level aluminum at pH 5.2 showed severe damage by day 4, with necrosis and fusion of secondary lamellae and hyperplasia and hypertrophy of mucous cells. Over the following 20 d, there was a continual process of repair with proliferation and hypertrophy of mucous cells. Qualitative analysis of gill samples plus physiology and mortality data collected in a companion study indicated progressive development (by day 10 onward) of increasing acclimation to Al. Quantitative analysis of gill samples on day 13 showed that mucous cell volume density had tripled and mucous cell area had doubled in Al-exposed fish compared with control fish. A lamellar fusion index showed evidence of fusion in Al-exposed fish by day 4 with recovery to nearly control levels by day 13. Physiological disturbances appear to be directly related to the histological changes observed in the gill epithelium. At the cellular level, changes in either mucous cell production and secretion or changes in mucus chemistry contribute, in part, to acclimation to Al.

L'analyse d'échantillons de branchies d'ombles de fontaine juvéniles acclimatés à de faibles teneurs en aluminium dans un milieu de pH 5,2 a révélé d'importantes lésions dès le quatrième jour: nécrose et fusion des lamelles secondaires ainsi que hyperplasie et hypertrophie des cellules muqueuses. Au cours des 20 jours suivants, on a observé une prolifération et une hypertrophie constantes des cellules muqueuses. Les résultats d'une analyse qualitative d'échantillons de branchies ainsi que des données sur la physiologie et le taux de mortalité recueillies dans le cadre d'une étude parallèle ont révélé l'apparition progressive, à partir du dixième jour, d'une acclimatation croissante à l'Al. L'analyse quantitative d'échantillons de branchies prélevés le treizième jour a révélé que la masse volumique des cellules muqueuses avait triplé et que la superficie des cellules muqueuses avait doublé chez les poissons exposés à l'Al par rapport aux poissons témoins. Un indice de fusion des lamelles a permis de noter une fusion dès le quatrième jour chez les poissons exposés à l'Al ainsi que leur rétablissement, dès le treizième jour, presque jusqu'au niveau observé chez les témoins. Les perturbations physiologiques semblent être directement liées aux modifications histologiques observées dans l'épithélium des branchies. Au niveau cellulaire, des modifications dans la production et la secrétion de cellules muqueuses ou de la chimie du mucus contribuent en partie à l'acclimatation à l'Al.

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Many morphometric and physiological studies indicate that low pH and elevated aluminum primarily cause damage at the gills. Many structural changes associated with these conditions have been described. After acute exposure of brook trout (*Salvelinus fontinalis*) to pH 5.2 or lower, Daye and Garside (1976) reported lifting of the outer epithelium from the pillar cells on the secondary lamellae, as well as some hypertrophy of filamental mucous cells. Fathead minnows (*Pimephales promelas*) chronically exposed to pH 5.5 or 5.0 showed increased numbers of chloride cells and increases in apical pit formations in these cells (Leino and McCormick 1984). In field studies of acidified lakes, Chevalier et al. (1985) found increased numbers of chloride cells in brook trout in addition to hyperplasia of the primary filament.

When Al is added to acid exposures, histological damage in fish gills is similar to that reported from acid exposure alone, yet the effects are often more severe. Changes described with acid/Al exposure include increases in diffusion distances as the epithelium lifts, increases in numbers of chloride cells, the presence of dense cells (a chloride cell variant), and extensive necrosis and degeneration of gill tissue (Tietge et al. 1988; Evans et al. 1988; Ingersoll et al. 1990). In addition to the reported Al effects on gill structure, there have been reports of intracellular and extracellular Al deposits (Karlsson-Norgren et al. 1986; Youson and Neville 1987).

Furthermore, it has been established that low-level chronic exposure to acid and Al results in a reduction in toxicity to Al.

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This phenomenon is described as acclimation (Orr et al. 1986; Wood et al. 1988a, 1988b). Its significance is that fish are often exposed to low-level "background" Al concentrations in acidified waters. This exposure may confer increased resistance to short-term pulses of high levels of Al that may occur during episodes of snowmelt or rainfall (Orr et al. 1986; Siddens et al. 1986; Wood et al. 1988a, 1988b; McDonald and Milligan 1988).

The physiological changes described following acid/Al exposure (McDonald et al. 1991), as well as acid/Al acclimation probably result from structural alterations at the gills. However, no study has simultaneously addressed both physiological and histological changes associated with damage or acclimation. Thus the objectives of this study were to address simultaneously the physiological and histological changes that may occur over the course of acclimation to acid and Al. This paper presents the results of the histological observations.

Methods

Exposure Conditions

All tests were conducted at the Fish Physiology and Toxicology Laboratory, Laramie, WY. Juvenile brook trout were continuously exposed to three acclimation conditions in flowing artificial soft water for up to 42 d. Acclimation conditions included the following: pH 6.5, 0 Al (controls); pH 5.2, 0 Al; and pH 5.2, 150 μg Al/L (reduced to 75 μg/L on day 3). All calcium levels were at 25 μequiv/L. Exposure animals were juvenile brook trout (average weight 5 g) that had been softwater acclimated for at least 60 d. One tank was used for each exposure and each tank began with 400–600 fish. To assess the nature and time course of acclimation, fish were periodically sampled from each group for physiological and histological status or for challenge to lethal Al (1000 μg/L) (Fig. 1). Physiological measurements included body electrolytes, hematology, gill chemistry, and branchial Na^+ fluxes. Resistance to challenge was assessed on the basis of mortality and by the magnitude of physiological disturbances in survivors.

From day 0 to 24 of the acclimation experiment, 52 fish from each acclimation group were removed at each sampling time (days 1, 2, 4, 7, 10, 13, 17, and 24). Sixteen of these fish were used for measurements of Na^+ uptake, of which half were subsequently returned to their respective acclimation tank and the other half sacrificed for measurements of whole-body electrolytes. Another 10 fish were immediately sacrificed and a blood sample obtained by caudal puncture using a modified 100 μL Hamilton gas-tight syringe. Hematocrit and plasma protein were determined on this blood sample. The second gill arch from the right side was taken for histological examination and the remaining gill basket was weighed, frozen, and later digested for gill Al and sialic acid determination. A third group of 10 fish from each acclimation group was transferred to a single challenge tank (pH 5.2, nominal Al = 1000 μg/L) and cumulative mortalities recorded over time until all fish were dead. An estimate of the time to 50% mortality (ET50) was determined by log/probit analysis for each group exposed to the challenge. A fourth group of 16 fish from each acclimation group was challenged simultaneously with the same condition; survivors at 24 h were sacrificed for either (i) determinations of whole-body electrolytes and lactate or (ii) measurements of hematocrit, plasma protein, gill Al and sialic acid content, and gill histology.

A final challenge experiment was begun on day 28 of the acclimation period. Here, a total of 90 fish from each of the 6.5/0 Al and 5.2/Al groups were transferred in groups of 15 to one of six challenge exposures (Fig. 1). The challenge exposures were at pH 4.8/0 μg Al/L, 100 μg/L, or 300 μg/L and at pH 5.2/0 μg Al/L, 100 μg/L, or 300 μg/L. Fish were maintained for 2 wk under these conditions and cumulative mortalities recorded over time. At the end of the 2 wk, up to 10 survivors from each group in each tank were then sacrificed for measurements of whole-body electrolytes, hematology, gill Al, and gill histology. (For additional details on exposure and sampling protocols, see McDonald et al. 1991.)

Tissue Preparation

Five fish from each acclimation and challenge group were sampled for histopathology. Fish were stunned by a blow to the head. The second gill arch from the right side was excised and immediately placed in 2.5% buffered glutaraldehyde solution for at least 24 h. Tissues were then postfixed in buffered 1% osmium tetroxide, dehydrated in a graded series of ethanols, rinsed in propylene oxide, and embedded in an araldite epoxy resin. Longitudinal thin sections (1 μm) were cut using a Sorvall MT-2B microtome, mounted on glass slides, and stained with either 0.1% Azure B or Eriochrome Azurol B Al-specific stain (Denton et al. 1984; Humason 1979).

Qualitative Examination

Gills were qualitatively examined for general pathology. Special categories of examination included primary filament hyperplasia, structural integrity of secondary lamellar epithelium, evidence of degeneration or necrosis, fusion of primary or secondary lamellae, proliferation of undifferentiated cells, size and distribution of chloride and mucous cells, and infiltration of white blood cells.

Stereology and Morphometry

Point count stereological methods (Weibel 1979) were used to quantify volume density of pillar cells, undifferentiated cells, inner epithelial cells, pavement cells, chloride cells, mucous cells, dense cells, and white blood cells in the secondary lamellae. Volume density of lymphatic spaces and pillar cell-capillary network were also determined by point counting.

Tissues were examined directly on an Olympus BH-2 microscope with a 10 mm square lattice grid (Olympus Corp., Precision Instrument Div., Lake Success, NY) in the ocular. This grid superimposed a regular array of 121 points on the microscope image. The entire gill tissue was first mapped to delineate usable fields and then five random fields per secondary lamella were selected for analysis using a random number generator. The ocular was randomly rotated between each field to assure random placement of the grid over each field. Points falling on parameters of interest were tallied and volume density of each component was calculated by dividing total points for each category by the total number of points in the tally. A minimum of 1000 points were counted.

Mucous cell area and lamellar fusion were measured using a digitizing tablet (Houston Instruments) interfaced to an IBM-XT computer equipped with a morphometric software package (Bioquant, Rand M Biometrics) (Tietge et al. 1988). Lamellar fusion measurements were made by projecting a slide image onto a monitor and tracing lamellar lengths and perimeters using

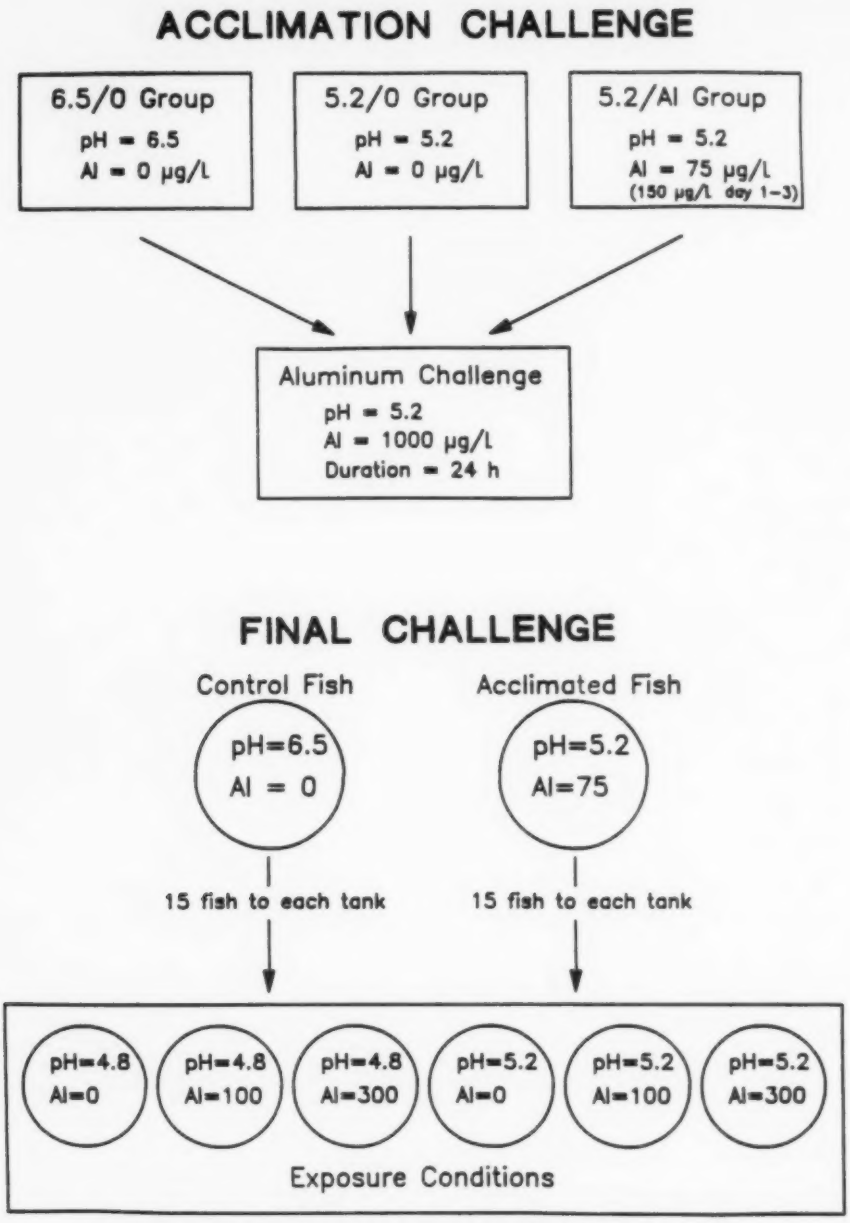

FIG. 1. Flow diagrams illustrating acclimation and final challenge conditions.

a digitizing tablet. Lengths (L1) of a random sample of secondary lamellae were traced down the center of the pillar capillary network from the intersection with the central venous sinus to the marginal channel. Perimeters (L2) of exposed epithelium were traced from the interlamellar midpoint of adjacent lamellae around the entire lamellae to the next adjacent interlamellar midpoint (Fig. 2). A lamellar fusion index was calculated by dividing L2/L1.

Statistical Analysis

Cell volume density (volume fraction of cell of interest relative to the entire gill) means were calculated for each fish (n = 5, except for controls where n = 4) and a final mean was calculated for each group. No less than 1000 points were tallied for each fish. Percents calculated were then transformed using the arc sine square root transformation. Mucous cell area means were not transformed. Summary statistics were generated and tested for homogeneity of variances using both Bartlett's and Hartley's test. All data were analyzed using analysis of variance ($p < 0.05$). If there were significant differences, pairwise comparisons were made using both Dunnett's and Tukey's test for mean comparisons. All analyses were performed using the TOXSTAT statistical computer program (Gulley et al. 1988).

FIG. 2. Diagram illustrating lamellar fusion index measurements. Measurements were taken by tracing a projected slide image using a digitizing tablet. Lamellar lengths (L1) were measured down the center of the pillar cell – capillary network (solid line) from the intersection with the central venous sinus of the marginal channel. Perimeter lengths (L2) of exposed epithelium were measured from the interlamellar midpoint of adjacent lamellae around an entire lamella to the next adjacent interlamellar midpoint (broken line). A lamellar fusion index was calculated by dividing L2/L1. (Diagram from Skidmore and Tovell 1972).

Results

Histology

Controls

Control gills (6.5/0 Al) exhibited normal gill architecture (Morgan and Tovell 1973) for all sampling periods with occasional exceptions (Fig. 3). Gill filaments consisted of a primary filament covered by undifferentiated cells and a surface layer of pavement epithelial cells. Projecting from the primary filament at regular intervals were leaflike structures known as secondary lamellae. These secondary lamellae were composed of a network of blood-filled spaces (capillary network) separated by pillar cells whose cell processes or flanges connected. Covering this pillar–capillary network was a thin basal lamina over which rested an inner and outer layer of epithelial cells. The inner layer of epithelium consisted of relatively undifferentiated cells found primarily with their nuclei above pillar cells. The outer epithelium consisted of a flattened layer of pavement cells, dome-shaped chloride cells, and mucous cells. Chloride cells were primarily located at the bases of the lamellae and were distinguished by prominent staining of mitochondria which gave the cytoplasm a granular appearance. Mucous cells, also found on the primary filament, stained darkly, as the mucopolysaccharide content was extremely basophilic. Also a feature of the normal gill was the presence of lymphatic spaces found primarily adjacent to chloride cells. A few control fish showed minor histological changes such as epithelial lifting and slight chloride cell proliferation, which was probably a result of holding stress (Fig. 3).

Exposure to pH 5.2/0 Al

Fish exposed to low pH in the absence of Al had primary filaments with slight hyperplasia of undifferentiated cells and some proliferation and hypertrophy of chloride cells by day 4 (Fig. 4A). The most prominent feature of the secondary lamellae was a lifting of the epithelium away from the basal lamina accompanied by an influx of white blood cells in the lymphatic spaces. Pavement epithelial cells were vacuolated and degenerating (Fig. 4A).

FIG. 3. Gills from control brook trout (6.5/0 Al group) illustrating (A) normal gill architecture observed in most sections as well as (B) minor pathology, probably indicative of holding stress, observed in occasional sections. In Fig. 3B, hypertrophic chloride cells have proliferated, occurring not only in the basal areas of the secondary lamellae but more distally as well. In some areas the outer epithelium is lifted away from the basal lamina. Note the size and location of a mucous cell. CN = capillary network, PC = pillar cell, PV = pavement cell, CC = chloride cell, EL = epithelial lifting, MC = mucous cell.

Exposure to pH 5.2/Al

To induce acclimation, fish were initially exposed to 150 µg Al/L. On day 3, the Al concentration was reduced to 75 µg/L due to unacceptably high mortality rates. The first samples examined histologically were taken on day 4, and these samples reflected damage of gills due to Al. Lesions in these gills were extensive and severe (Fig. 4B, 4C).

The primary filament and secondary lamellae were hyperplastic, with the proliferating cells being completely detached (desquamating) from the basal lamina and extended distally towards the outermost tips of the secondary lamellae (Fig. 4B). Although cells had lost their attachments to the basal lamina, some cells had maintained lateral connections which gave the appearance of an outer membrane or epithelium covering the tips of adjoining secondary lamellae (Fig. 4B, 4C). The only remaining discrete structure was the pillar cell – capillary net-

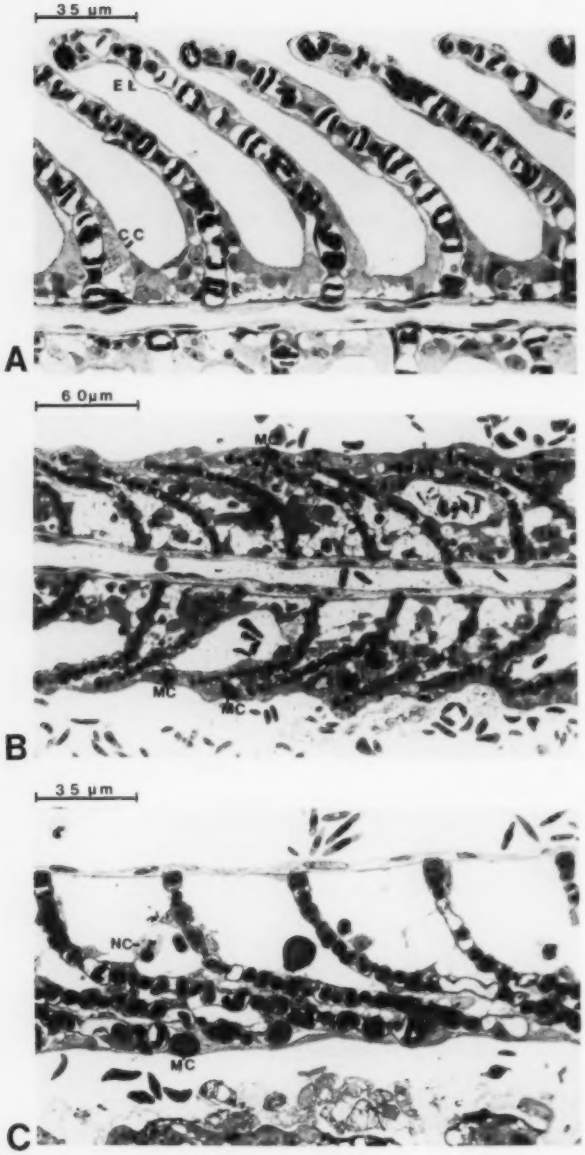

FIG. 4. Gills from brook trout acclimated for 4 d to (A) low pH (pH 5.2) and (B and C) low pH plus Al (pH 5.2/Al). In Fig. 4A, note chloride cell proliferation (CC) and epithelial lifting (EL) often associated with acid stress. In Fig. 4B, the primary filament is extensively hyperplastic and the proliferating cells are detached from the basal lamina and located at the outermost tips of the secondary lamellae. Note the appearance of an "outer membrane" or epithelium covering the tips of adjacent secondary lamellae. There is extensive hyperplasia and hypertrophy of mucous cells (MC). Necrotic and degenerating aggregates of cells fill the interlamellar regions. In Fig. 4C, the pillar–capillary network, surrounded by the basal lamina, is the only structure remaining intact on the secondary lamellae. Mucous cells are extremely hypertrophic and have proliferated to the tips of the secondary lamellae. Necrotic cells (NC) are seen in the interlamellar regions.

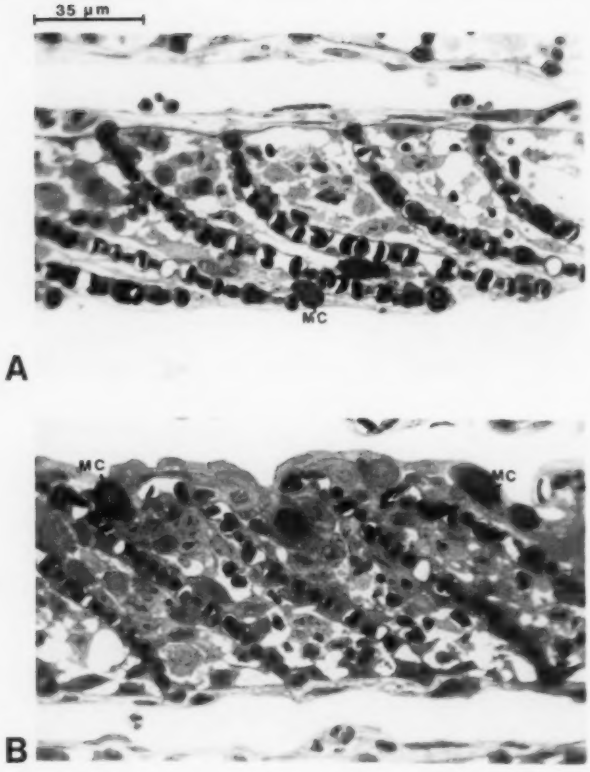

FIG. 5. Gills from brook trout exposed to low pH and Al (pH 5.2/Al) from the acclimation experiment sampled on days 7 and 10. (A) Gill sampled on day 7 showing severe damage to the secondary lamellae. Loose aggregates of degenerate and necrotic cells fill the interlamellar regions. Remaining epithelial cells are distally located and appear to cover the tips of the secondary lamellae. Mucous cells (MC) are hyperplastic and hypertrophic. (B) Gill from fish sampled on day 10. Proliferating undifferentiated cells fill the interlamellar regions resulting in fused secondary lamellae. Hyperplastic mucous cells are extremely hypertrophic.

work surrounded by a basal lamina (Fig. 4C). Necrotic and degenerating aggregates of cells, undifferentiated proliferating cells, and fluid filled the interlamellar spaces (Fig. 4B, 4C).

Mucous cells had proliferated throughout the gill filament and the secondary lamellae. These cells were extremely hypertrophic and they were commonly located on the outer layer of remaining cells that had formed at the distal ends of the secondary lamellae (Fig. 4B, 4C). Control gills had few mucous cells on the gill filaments and rarely any on the secondary lamellae.

Identifiable chloride cells did not appear to proliferate but were hypertrophic and often degenerate. However, undifferentiated cells were proliferating, and some of these may have been developing chloride cells. Pillar cells seemed least affected, although they appeared to be slightly swollen.

Subsequent samples taken on days 7, 10, 13, and 24 revealed a continuing process of repair (Fig. 5, 6). Desquamation of proliferated cells continued to abate, with masses of connected cells gradually replacing what had been isolated masses of aggregated cells (Fig. 5, 6). Lamellae that had been fused by these masses gradually became separated as the proliferation

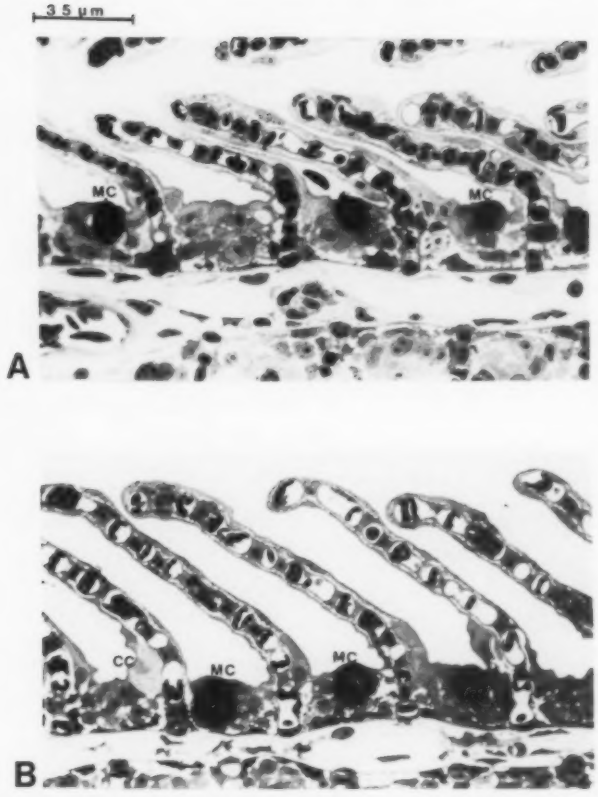

FIG. 6. Gills from brook trout exposed to low pH and Al (pH 5.2/Al) from acclimation experiment sampled on day 13 and 24. (A) Gill from fish sampled on day 13. Proliferation of undifferentiated cells has subsided with hyperplasia restricted to the primary filament. Mucous cells (MC) remain hyperplastic and hypertrophic. There is slight lifting of the epithelium. (B) Gill from fish sampled on day 24. The secondary lamellae show evidence of extensive repair. Mucous cells remain hypertrophic but are confined to the primary filament. Hypertrophic chloride cells (CC) are apparent.

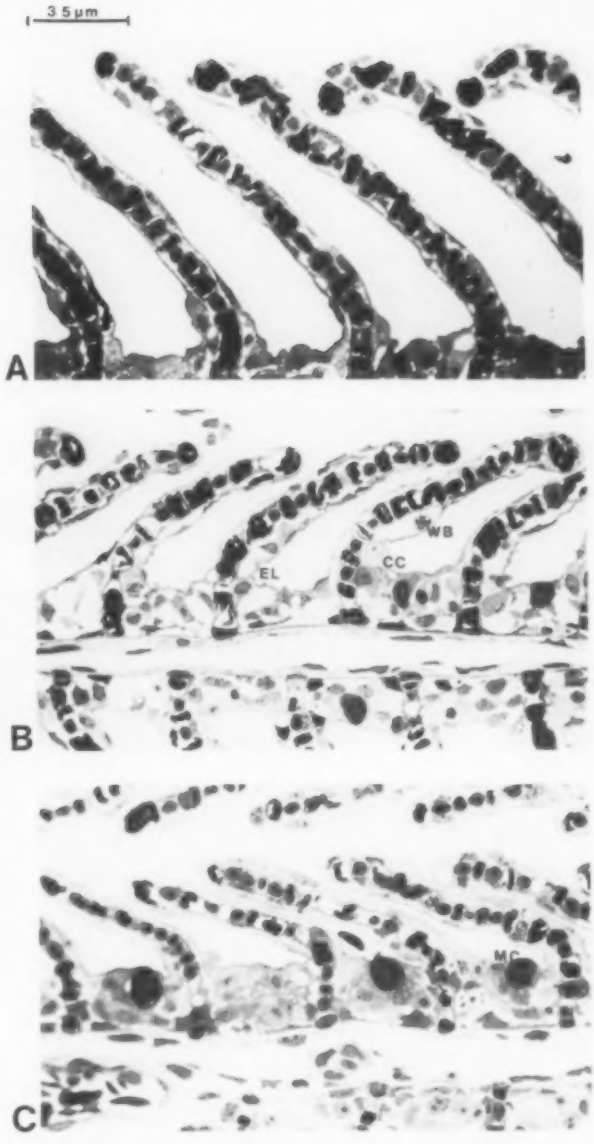

FIG. 7. Comparison of brook trout gills from control (pH 6.5/0 Al), low pH (pH 5.2/0 Al), and low pH plusAl (pH 5.2/Al) exposure sampled on day 13 of the acclimation experiment. (A) Secondary lamellae from control gill showing a flat epithelium surrounding a pillar–capillary network. Note the absence of hypertrophic mucous cells or epithelial lifting. (B) Primary filament from low pH exposed fish showing extensive hyperplasia. The epithelium of the secondary lamellae is lifted away from the basal lamina (EL). Chloride cells (CC) are hypertrophic and white blood cells (WB) are present in the lymphatic spaces. (C) The primary filament and secondary lamellae from a low pH plus Al exposed fish shows proliferation of undifferentiated cells and hypertrophy and hyperplasia of mucous cells (MC). Epithelium of the secondary lamellae is thickened.

subsided (Fig. 6A). By the end of the acclimation experiment (day 24), undifferentiated cells of the primary filament as well as some cells in the proximal portions of the secondary lamellae remained hyperplastic (Fig. 6B). The distal portions of the secondary lamellae had relatively normal epithelium with the exception of mucous cells described below. Necrotic and degenerating cells decreased over time.

Mucous cells remained a prominent and persistent feature throughout the acclimation period in the Al-exposed fish (5.2/Al group). Although these cells appeared to be less hypertrophic at the end of the acclimation period, they were still proliferating. They occurred throughout the primary filament (Fig. 6B) and secondary lamellae.

Near the end of the acclimation period, chloride cells appeared to increase in number, suggesting that many of the undifferentiated proliferating cells, observed on day 4, may have been developing chloride cells. In addition, these and the surrounding cells had highly folded margins. There was a noticeable increase in apical pit formation as well as chloride cell epithelial margins (Fig. 6B). The folding may have been the result of displacement and/or pressure from the surrounding hyperplastic cells.

Comparison of gills from the three acclimation groups on day 13 illustrated distinct differences (Fig. 7). Control groups

(6.5/0 Al) (Fig. 7A) appeared normal, while the low pH groups (5.2/0 Al) showed evidence of acid stress (Fig. 7B). In the low-pH group, lifting of the epithelium was common as were white blood cells in the lymphatic spaces. Mucous cells were scarce. In contrast, the low pH plus Al groups (5.2/Al) (Fig. 7C) had many hypertrophic mucous cells along the primary filament and occasionally on the secondary lamellae. In addition, undifferentiated cells had proliferated and some pavement cells of the secondary lamellae were thickened and vacuolated.

Challenge gills

Gill samples were taken from surviving fish after each Al challenge. By day 13, mortality and physiological observations indicated a reduced response to the challenge in fish previously exposed to low-level Al (75 μg/L) (McDonald et al. 1991). Fish from the control (6.5/0 Al) and 5.2/0 Al groups showed desquamation of the epithelium from both the primary filament and the secondary lamellae with massive necrosis and extracellular edema after exposure to pH 5.2, 1000 μg Al/L (Fig. 8A, 8B). Often only a few epithelial cells remained attached at the most distal portions of the secondary lamellae near the marginal channels. Any remaining epithelial cells were degenerate or necrotic. Only the pillar–capillary network remained intact (Fig. 8A, 8B).

Conversely, gills from acclimated fish, those continuously exposed for 13 d to pH 5.2, 75 μg Al/L, were dramatically different. These gills still showed evidence of the damage induced by initial low-level Al, but the effects of Al challenge were minimal (Fig. 8C). There were areas of fusion and proliferation of undifferentiated cells with hyperplastic mucous and chloride cells. Epithelial cells, although swollen and vacuolated, remained attached to the basal lamina (Fig. 8C).

Fish examined after challenge on day 24 were similar to those seen on day 13, especially the control (6.5/0 Al) and low-pH (5.2/0 Al) groups (Fig. 9A, 9B). Acclimated fish (those previously exposed to low-level Al for 24 d), although affected by the challenge, showed lamellae still hyperplastic but with few areas of fusion (Fig. 9C). Hyperplasia of the primary filament and a preponderance of mucous cells were the most noticeable features. There was some proliferation and hypertrophy of chloride cells, but this was mostly confined to the proximal portions of the secondary lamellae. Distal portions of the lamellae appeared nearly normal with the exception of some areas of epithelial lifting and dilation of lymphatic spaces.

Final challenge groups

The only fish with sufficient survivors for histology sampling were those challenged at pH 5.2 at 0 and 100 μg Al/L for both controls (6.5/0 Al) and acclimated (5.2/Al) fish. At pH 5.2, 300 μg Al/L, only previously acclimated fish were sampled, as there were no survivors in the control group.

Gills from acclimated 5.2/Al fish in Al challenge appeared structurally similar to those gills taken at the end of the acclimation period, but with evidence of more repair. Mucous cells were prominent but not hypertrophic. They were evident on both the primary filament and the secondary lamellae (Fig. 10B). Hyperplasia of the primary filament was present but reduced. Chloride cell proliferation was slight and there appeared to be an increase in the number of phagocytic white cells. In some areas, the epithelium was lifted and the lymphatic spaces were dilated. Gills from the control group (6.5/0 Al) after the 5.2/0 Al challenge showed evidence of acid stress with epithelial lifting as the primary feature (Fig. 10A).

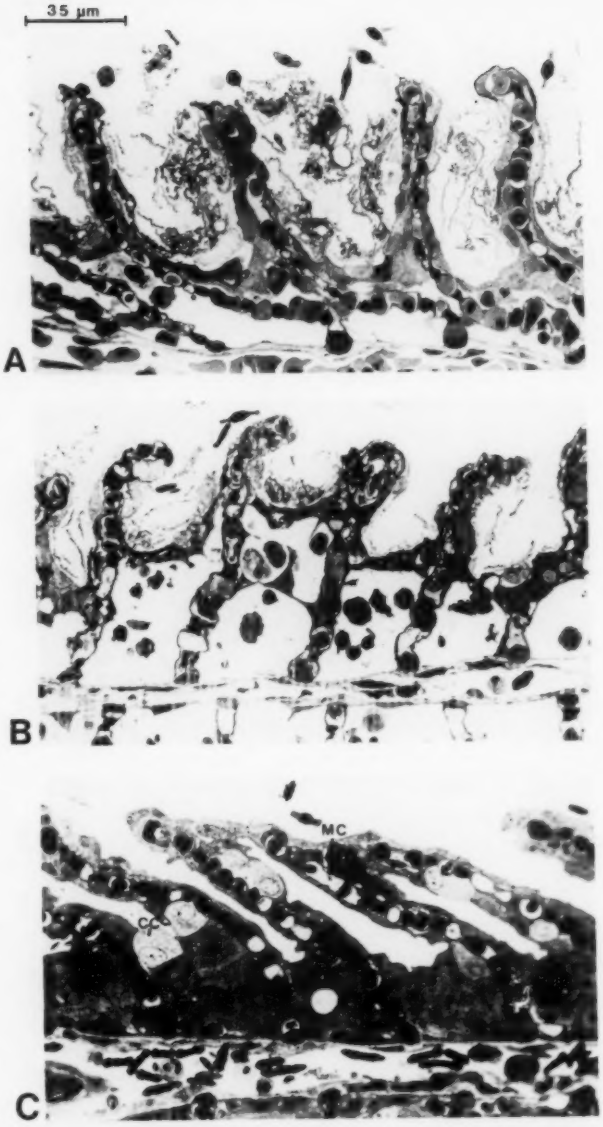

FIG. 8. Comparison of brook trout gills from control (pH 6.5/0 Al), low pH (pH 5.2/0 Al), and low pH plus Al (pH 5.2/Al) exposure after challenge with 1000 μg Al/L for 24 h on day 13. (A) Control gill (6.5/0 Al) showing necrosis of the lamellar epithelium. Only the pillar–capillary network is identifiable. (B) Low pH gill (5.2/0 Al) also showing necrosis of the lamellar epithelium. Blood spaces are distended and necrotic cells and debris fill the interlamellar spaces. (C) Low pH plus Al (5.2/Al) gill showing considerably less damage than control or low pH fish (Fig. 8A, 8B). There is proliferation of undifferentiated cells and some areas of lamellar fusion. Mucous cells (MC) and chloride cells (CC) are hypertrophic.

In the pH 5.2/100 Al final challenge, there were distinct differences between gills from control fish and those previously acclimated to pH 5.2 and 75 μg Al/L. Control fish showed hyperplasia of the primary filament (Fig. 11A) with occasional areas of fusion at the tips of the secondary lamellae. Mucous cells had proliferated and were hypertrophic and generally

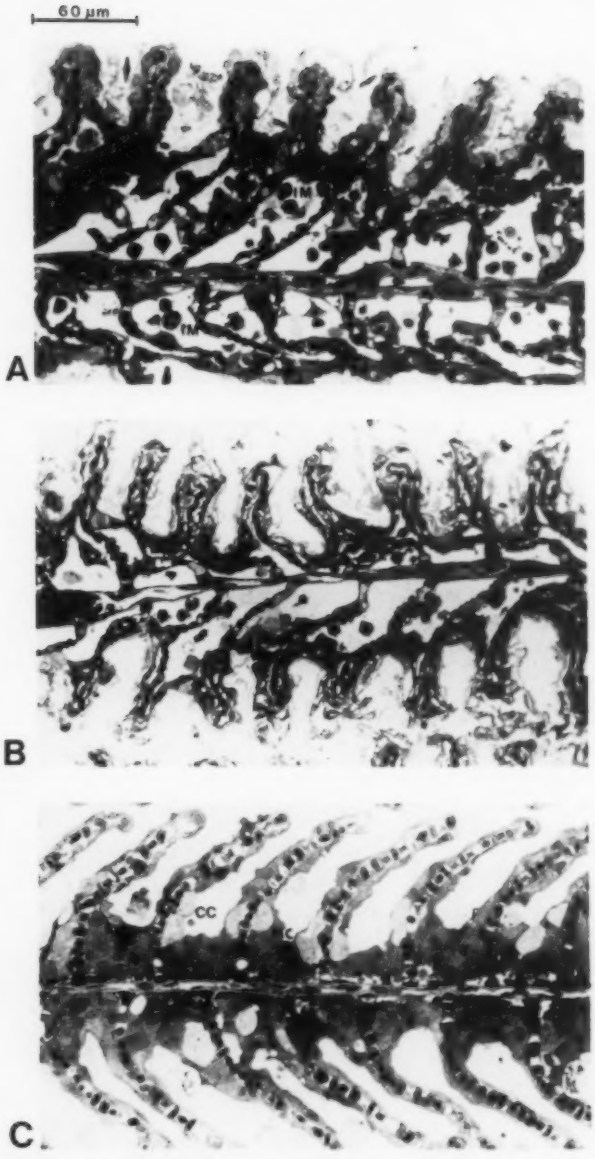

FIG. 9. Comparison of brook trout gills from control (pH 6.5/0 Al), low pH (pH 5.2/0 Al), and low pH plus Al (pH 5.2/Al) exposure after challenge with 1000 µg Al/L for 24 h on day 24 of the acclimation period. (A) Gill from control fish (pH 6.5/0 Al) showing desquamation of the epithelium from both the primary filament and the secondary lamellae. White blood cells, immature mucous cells (IM), and necrotic debris fill the fluid-filled interlamellar regions. (B) Gill from low pH fish (pH 5.2/0 Al) showing cellular necrosis. Necrotic debris fills the interlamellar spaces. (C) Gill from low pH plus Al (pH 5.2/Al) fish is dramatically less damaged than controls. There is hyperplasia of the primary filament and proliferation and hypertrophy of chloride cells (CC) and mucous cells (MC). Although most portions of the secondary lamellar are nearly normal, epithelial lifting is evident.

located at the bases of the secondary lamellae. Chloride cells were extremely hypertrophic and had proliferated throughout the secondary lamellae. The epithelium of the secondary lamellae appeared lifted, and pavement cells appeared swollen.

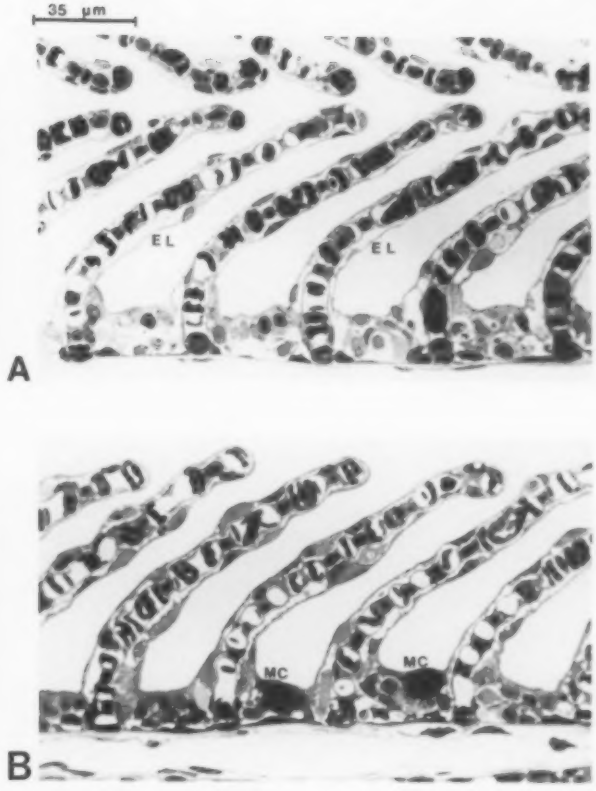

FIG. 10. Comparison of brook trout gills from control fish (pH 6.5/0 Al) with gills from "acclimated" fish (those previously exposed to pH 5.2, 75 µg Al/L for 24 d) when challenged with low pH (5.2/0 Al) for 14 d. (A) Gill from unacclimated fish (6.5/0 Al) showing evidence of acid stress with prominent epithelial lifting (EL). There is slight hyperplasia and edema of the primary filament. (B) Gills from previously acclimated fish (pH 5.2/Al) showing hyperplasia of the primary filament. Mucous cells (MC) are present but less hypertrophic.

In contrast, fish previously acclimated to pH 5.2 and 75 µg Al/L showed minimal effects. Again, the most prominent feature was the hypertrophic and proliferated mucous cells, although in this group they were confined to the primary filament and basal portions of the secondary lamellae. The distal portions of the secondary lamellae were nearly normal, although slightly thickened with numerous phagocytic white cells in the lymphatic spaces (Fig. 11B).

Gills from the acclimated fish that survived the final pH 5.2/300 Al challenge showed definite lesions, although these were not nearly as severe as those from fish examined (initially) during the acclimation experiment (Fig. 4B, 4C). In the gills from acclimated fish after the 14-d challenge (Fig. 11C), extreme proliferation and hypertrophy of chloride cells was a dominant feature with increased apical pit formation. Hyperplasia of the primary filament and areas of degeneration were common. Mucous cells were hypertrophic but were not as numerous as in other experiments. The epithelium of the secondary lamellae was degenerate with many vacuolated and swollen pavement cells.

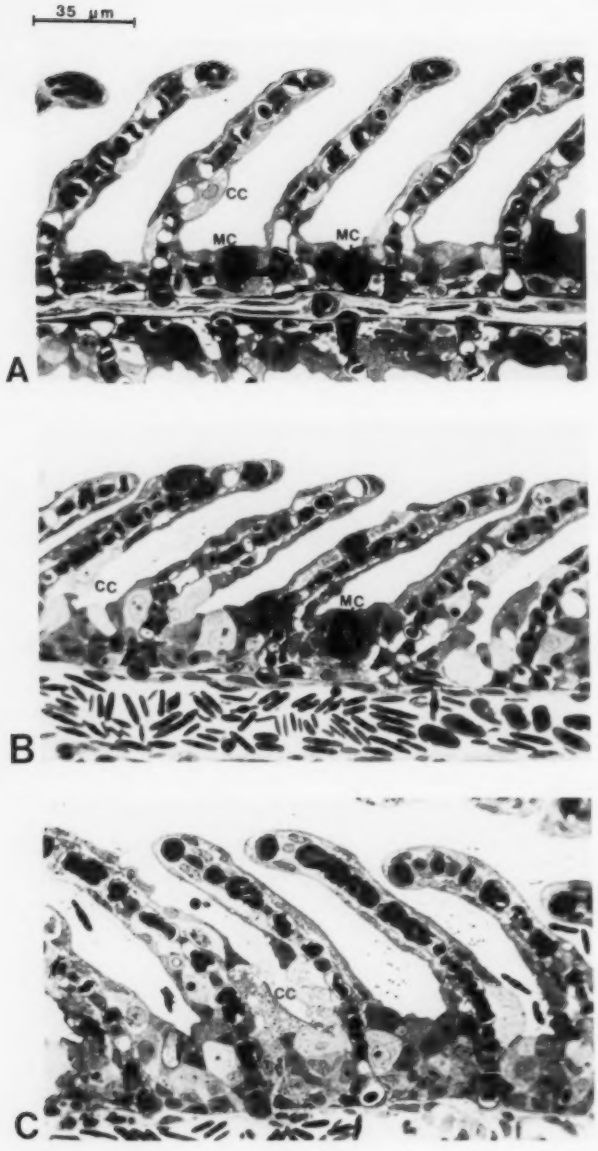

FIG. 11. Comparison of brook trout gills from control fish (pH 6.5/0 Al) with gills from "acclimated" fish (those previously exposed to pH 5.2, 75 µg Al/L for 24 d) when challenged with low pH and Al (5.2, 100 µg Al/L) for 14 d and with gills from "acclimated" fish challenged with 5.2, 300 µg Al/L for 14 d. (A) Gill of challenged unacclimated (6.5/0 Al) fish showing hyperplasia of the primary filament and proliferation and hypertrophy of mucous cells (MC) and chloride cells (CC). The epithelium of the secondary lamellae is lifted. (B) Gill of challenged previously acclimated (5.2/Al) fish showing minor hyperplasia of the primary filament. Secondary lamellar epithelium is nearly normal. Mucous cells remain hypertrophic. (C) Gill of challenged acclimated (5.2/Al) fish showing hyperplasia of the primary filament and hypertrophy and hyperplasia of chloride cells. The epithelium of the secondary lamellae is thickened and white blood cells are present in the lymphatic spaces.

Morphometry

By day 13 of the acclimation period, physiological and mortality data indicated that fish acclimated to pH 5.2, 75 µg Al/L exhibited a reduced response to an Al challenge over controls and pH 5.2/0 Al fish. Therefore, samples taken from all three acclimation groups on this day were selected for a quantitative morphometric analysis.

Exposure to pH 5.2/Al

Gills from fish acclimated to pH 5.2 and 75 µg Al/L had significantly higher ($p < 0.05$) volume densities for undifferentiated cells and mucous cells than did those from fish from other groups (Fig. 12). Volume density of mucous cells had nearly doubled from 0.09 to 1.8%, while that of undifferentiated cells, an indication of the proliferative response, tripled from 7.5 to 23.3%. Mucous cell area, a measure of hypertrophy, was significantly increased, being nearly twice that of controls or 5.2/0 Al fish (Fig. 13). Volume densities of chloride pillar cells were significantly decreased (Fig. 12). There were no significant differences seen in any other cell categories (capillary space, pavement cells, basal cells, empty lymphatic space (ELS), white blood cells, dense cells, degenerated or necrotic cells, and unknown cells).

Direct measurements of diffusion distance and lamellar thickness could not be made on these tissues because of extensive fusion of the lamellae in Al-exposed fish. In these cases, a lamellar fusion index was calculated as a relative measure of damage reflecting some deterioration in respiratory ability. In control fish with little or no hyperplasia or fusion, the expected ratio of exposed perimeter to length of lamellae in a cross-sectional plane would be nearly 2:1 (or 2). Therefore, in fish with areas of extensive fusion, this ratio would be less than 2 , indicating a reduced amount of epithelium exposed to the water and available for gas exchange. At the height of damage (gills sampled at day 4), this index ranged from 0.408 to 0.912, indicating massive fusion and loss of respiratory capacity. By the time the fish had acclimated (by day 13), this index had increased to that of control fish with no significant differences among groups on day 13 (one-way ANOVA, Tukey's multiple comparison, $P > 0.05$) (Table 1).

Exposure to 5.2/0 Al

In most categories, pH 5.2/0 Al fish did not differ from controls. The exceptions were the greater amount of empty lymphatic space in this group over controls and that of Al-exposed fish, and significantly fewer pillar cells than controls (Fig. 12). The increase in empty lymphatic space is misleading because dilation of the lymphatic space appeared more severe in Al-exposed fish but was often filled with degenerating aggregates of cells, leukocytes, and necrotic debris, and "hits" in these areas rarely encountered actual "empty" space. There were also significantly fewer undifferentiated cells in this group relative to Al-exposed fish (5.2/Al).

Al Staining

Al staining was used to confirm the presence of Al on Al-exposed gills. Al was often seen in a mucoid microlayer on the surface of the epithelium as well as in dense accumulations of mucus in the interlamellar regions. As expected, challenge fish showed qualitatively greater amounts of mucus and Al than prechallenge fish, but no attempt was made to quantify differences between groups.

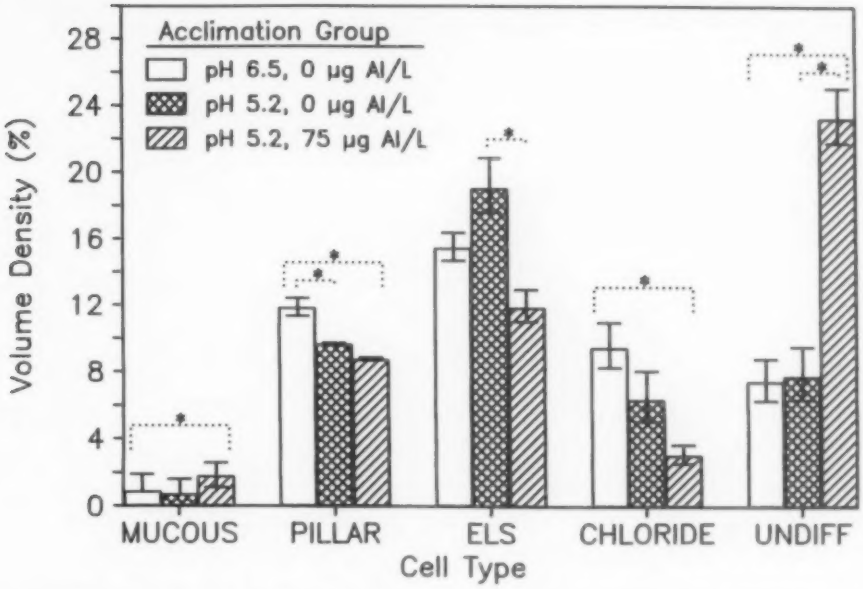

FIG. 12. Histogram comparing cell volume densities of mucous, pillar, chloride, undifferentiated cells, and empty lymphatic space (ELS) among acclimation groups on day 13. Bars represent mean values expressed as percents. Lines at the top of each bar show ± SEM; asterisks indicate significance difference at $p < 0.05$.

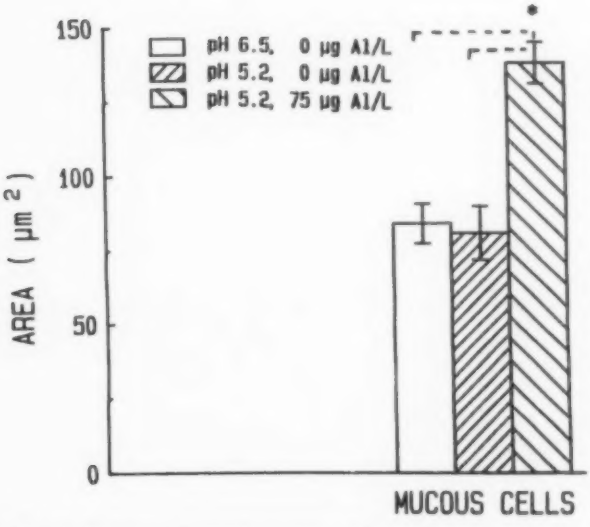

FIG. 13. Histogram comparing mucous cell area among acclimation groups on day 13. Bars represent mean values for mucous cell areas. Lines at the top of each bar show ± SEM; asterisks indicate significant difference at $p < 0.05$.

TABLE 1. Mean values for lamellar fusion indices (L2/L1). n = sample size; SD = standard deviation. *Significantly different from other groups (Tukey's multiple comparison, $p = 0.05$).

Exposure conditions	Day	n	L2/L1	SD
6.5, 0 Al	13	5	2.00	0.033
5.2, 0 Al	13	5	2.01	0.074
5.2, 75 Al	13	5	1.67	0.463
5.2, 75 Al	4	4	0.62*	0.216

Discussion

Exposure of juvenile brook trout to sublethal Al concentrations at low pH provokes profound changes in gill morphology, changes that are much more marked than those provoked by the same low pH alone. These morphological changes can be characterized as a two-phase response: rapid damage (reaching a peak at or before 4 d) followed by gradual repair. The repair phase was almost complete by 24 d of Al exposure. While the repair process was obvious, there were subtler acclimation-related changes as well, for fish challenged after acclimation to low levels of Al showed much less morphological damage than did unacclimated fish.

Since we have extensive physiological data for the same fish that were examined histologically (McDonald et al. 1991), it is now possible to put these profound structural changes at the gills into a physiological context and vice versa.

Damage Phase

Earlier studies on brook trout (Booth et al. 1988; McDonald and Milligan 1988; Wood et al. 1988a, 1988b) indicated that the physiological response to Al exposure was a two-phase response: an initial shock phase and a recovery phase. The "shock" phase of Al exposure is typically characterized by large diffusive ion losses plus an inhibition of active ion uptake and interference with oxygen and carbon dioxide movement across the gills (Booth et al. 1988; Wood et al. 1988a, 1988b). The net effect is a marked net loss of electrolytes across the

gills, an associated contraction of the blood volume, and internal hypoxemia and acidosis, the combination of which may lead to death. Fish that die of Al exposure never escape from this phase, but surviving fish show some recovery in which ion balance is reestablished and may, in fact, show some net ion gain. For the fish in the present study, the depression in body Na^+ reached a peak at day 4 (fig. 2A in McDonald et al. 1991) and then began to recover gradually. Hence the gills examined at day 4 probably represent the maximum gill damage experienced by surviving fish.

Histologically, the damage at day 4 consisted of extensive necrosis, desquamation of the gill epithelium, and lamellar fusion (Fig. 4B, 4C). The lamellar fusion was so severe that lamellar surface area could have been reduced by as much as 75%. This would impair oxygen delivery to the tissues as was indicated by an increase in body lactate levels relative to controls (Fig. 2E in McDonald et al. 1991). The increase in lactate was about 80% of the increase seen following exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) of similar size (M. Scarabello and C. M. Wood, McMaster University, Hamilton, Ont., unpubl. data). It is, therefore, likely that these fish had very little reserve to increase their metabolism or activity level and that their body glycogen reserves would have been depleted by anaerobic metabolism.

Other physiological correlates of the Al damage were the accumulation of Al on the gills and corresponding reduction in gill sialic acid (McDonald et al. 1991). Sialic acid is the major cationic component of gill mucus (Harris et al. 1973). Staining indicated that Al was primarily confined to the mucus layer and mucous cells, thus indicating probable Al binding to sialic acid residues. The corresponding histological response was extensive proliferation and stimulation of mucous cells which were fully evident in fish first examined at day 4 (Fig. 4B, 4C). These proliferated cells probably became functional and hypersecretory before this time, however, as gill Al peaked at day 1 of exposure and subsequently declined (fig. 2F in McDonald et al. 1991). By day 4, gill Al levels had fallen to half of that reported after 24 h. Hypertrophy of mucous cells thus appeares to be the first defense against Al exposure, and the combination of hypertrophy with the decline in sialic acid content of the tissue (McDonald et al. 1991) suggests either a change in the chemical composition of mucus with time or a considerable increase in mucus turnover in the gills. In any case, as gill Al levels fell, sialic acid content returned to preexposure levels and mucous cell hypertrophy persisted. The fact that gill Al levels thereafter remained low, despite the presence of Al in the water, suggests that elevated mucus secretion and sloughing was acting as a mechanism to prevent Al damage or accumulation.

Repair Phase

By Day 13, Al-exposed fish had nearly achieved physiological homeostasis. While they had not recovered completely, they showed no further deterioration in body electrolytes or in hematocrit (fig. 2 in McDonald et al. 1991). By this time, a remarkable degree of repair of the gill damage had taken place (Fig. 7C). There was considerable decrease in fused lamellae, and a corresponding decline in body lactate levels, indicating a return in gas transfer capacity towards normal levels. Necrosis and degeneration of cells had become rare, and the significant increases in mucous cell and undifferentiated cell densities relative to controls (Fig. 7C) were suggestive of a highly dynamic, rapidly changing epithelium. In previous studies, fish gills have been shown to respond to Al exposure with a rapid hypertrophy and hyperplasia of chloride cells (Evans et al. 1988; Youson and Neville 1987; Karlsson-Norgren et al. 1986; Tietge et al. 1988). The apparently delayed response in the present study may have occurred because the chloride cell proliferation was masked by the severity of the initial damage and subsequent repair. As pointed out earlier, many of the proliferating cells were not identifiable but may have been developing chloride cells. By day 24, chloride cells had increased in number (visual estimate) and appeared more folded with increased apical pit formations (Fig. 6B). These changes are thought to increase surface area for ion uptake to compensate for ion loss (Chevalier et al. 1985; Leino and McCormick 1984; Youson and Neville 1987). We did not see the appearance of "dense cells," a chloride cell variant reported by Tietge et al. (1988). This may have been because our study was 24 d in duration as compared with 147 d in that study. The apparently slow chloride cell response, in fact, correlated well with the physiological response (McDonald et al. 1991). Gill Na^+ transport was significantly impaired by sublethal Al exposure (fig. 5A in McDonald et al. 1991); it was maximally depressed by day 4 and then gradually recovered reaching almost normal levels by day 13.

Acclimation to Al

By day 13, the Al-exposed fish had not only achieved physiological homeostasis, but even more importantly had developed significant resistance to otherwise lethal Al concentrations (fig. 6 in McDonald et al. 1991). Fish were challenged with 1000 μg Al/L on three occasions between day 13 and 24, and in each case, the Al-acclimated fish exhibited longer survival times (i.e. increased ET50) and much smaller depressions in body electrolytes (fig. 2 in McDonald et al. 1991). Structural correlates of the increased resistance of acclimated fish were clearly seen in the histological results of the present study (Fig. 8, 9) where acclimated animals showed much attenuated gill morphological response to an otherwise lethal Al challenge. The implication of this finding is that Al is, in some way, chemically complexed before it is able to react with binding sites on the gills. The hypertrophy of mucous cells and proliferation of undifferentiated cells may be of key importance here, for these are the major histological differences between acclimated and unacclimated animals. However, the protective effect of preexposure did not arise from an elevated gill mucous content, for as pointed out earlier, the hypertrophy of mucous cells was not accompanied by an increase in sialic acid content; acclimated and unacclimated animals were virtually identical in this respect by day 7. Rather, it seemed more likely that there was a change in the chemistry of the mucus such that sialic acid content was not representative of mucus content and/or an increased turnover and sloughing rate of the mucus.

In a related study in rainbow trout, Reid et al. (1991) established that sublethal Al acclimation led to significant changes in the cation-binding properties of the gills. Specifically, these consisted of an increased binding affinity for Ca^{2+}, a decreased binding affinity for Al, and a decline in the potency of Al at displacing Ca^{2+}. These changes would contribute to reducing the reactivity of Al at the gill surface. These changes may result entirely from mucous cell hypertrophy and increased mucus production but there may also be changes at the outer epithelial membrane which are below the resolution of histological tech-

niques used in this study. Clearly some of the responses of the gills are too subtle to be resolved by the physiological and morphological techniques employed here. Therefore, a detailed examination of the ultrastructural changes involved in damage and repair processes should also be included in future analyses of branchial responses to environmental stress.

Whatever the mechanistic explanation for acclimation to Al, neither physiological measures (McDonald et al. 1991) nor histological observations provide definite markers for the exact time during exposure when increased resistance first occurs. Acclimation is likely a graded rather than a sudden process. There appears to be some threshold level, not detected morphologically, that must be reached in the compromise between protective measures such as increased production and secretion of mucus versus fusion of lamellae and the decreased ability for gas exchange. Histological observations suggest that this continuum of repair and alteration is gradual and dependent on initial damage due to the toxicant.

In addition, one must be careful in defining "benefit" in terms of the life of the fish. Surely in waters where spring and fall pulses of low pH and Al occur, there is an advantage for a fish with altered gill morphology such as that seen in this study. Our studies have demonstrated that acclimation to low pH and Al increases resistance to an otherwise lethal Al challenge. However, between these Al pulses, or in areas where no pulses occur, the morphological changes described for acclimated fish would inhibit normal activity. Increased diffusion distances and fused lamellae restrict oxygen transfer ability which would limit aerobic activity. It has yet to be determined how much and for how long the changes resulting in acclimation persist.

Acknowledgements

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References

Booth, C. E., D. G. McDonald, B. P. Simons, and C. M. Wood. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1563–1574.

Chevalier, G., L. Gauthier, and G. Moreau. 1985. Histopathological and electron microscopic studies of gills of brook trout, *Salvelinus fontinalis*, from acidified lakes. Can. J. Zool. 63: 2062–2070.

Daye, P. G., and E. T. Garside. 1976. Histopathologic changes in surficial tissues of brook trout, *Salvelinus fontinalis* (Mitchell), exposed to acute and chronic levels of pH. Can. J. Zool. 54: 2140–2155.

Denton, J., A. Freemont, and J. Ball. 1984. Detection and distribution of aluminum in bone. J. Clin. Pathol. 37: 136–142.

Evans, R. E., S. B. Brown, and T. J. Hara. 1988. The effects of aluminum and acid on the gill morphology in rainbow trout, *Salmo gairdneri*. Environ. Biol. Fishes 22: 299–311.

Gulley, D. D., A. M. Boelter, and H. L. Bergman. 1988. TOXSTAT. University of Wyoming, Laramie, WY.

Harris, J., A. Watson, and S. Hunt. 1973. Histochemical analysis of mucous cells in the epidermis of brook trout, *Salmo trutta* L. J. Fish Biol. 5: 345–351.

Humason, G. 1979. Animal tissue techniques. 4th ed. W. H. Freeman and Co., San Francisco, CA.

Ingersoll, C. G., D. D. Gulley, D. R. Mount, M. E. Mueller, J. D. Fernandez, J. R. Hockett, and H. L. Bergman. 1990. Aluminum and acid toxicity to two strains of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 47: 1641–1648.

Karlsson-Norgren, L., I. Bjorklund, O. Ljungberg, and P. Runn. 1986. Acid water and aluminum exposure: experimentally induced gill lesions in brown trout, *Salmo trutta* L. J. Fish Dis. 9: 11–25.

Leino, R., and H. McCormick. 1984. Morphological and morphometrical changes in chloride cells of the gill of *Pimephales promelas* after chronic exposure to acid water. Cell Tissue Res. 236: 121–128.

McDonald, D. G., and C. L. Milligan. 1988. Sodium transport in the brook trout, *Salvelinus fontinalis*: effects of prolonged low pH exposure in the presence and absence of aluminum. Can. J. Fish. Aquat. Sci. 45: 1606–1613.

McDonald, D. G., C. M. Wood, R. G. Rhem, M. E. Mueller, D. R. Mount, and H. L. Bergman. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). I. Physiology. Can. J. Fish. Aquat. Sci. 48: 2006–2015.

Morgan, M., and P. Tovell. 1973. The structure of the gill of the trout *Salmo gairdneri* (Richardson). Z. Zellforsch. 142: 147–162.

Orr, P. L., R. W. Bradley, J. B. Sprague, and N. J. Hutchinson. 1986. Acclimation-induced change in toxicity of aluminum to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 243–246.

Reid, S. D., D. G. McDonald, and R. R. Rhem. 1991. Acclimation to sublethal aluminum: modifications of metal – gill surface interactions of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1996–2005.

Siddens, L. K., W. K. Seim, L. R. Curtis, and G. A. Chapman. 1986. Comparison of continuous and episodic exposure to acidic, aluminum-contaminated waters of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 43: 2036–2040.

Skidmore, J., and P. Tovell. 1972. Toxic effects of zinc sulphate on the gills of rainbow trout. Water Res. 6: 217–230.

Tietge, J. E., R. D. Johnson, and H. L. Bergman. 1988. Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long-term exposure to acid and aluminum. Can. J. Fish. Aquat. Sci. 45: 1643–1648.

Weibel, E. R. 1979. Stereological methods. Vol. 1: Practical methods for biological morphometry. Academic Press, New York, NY. 415 p.

Wood, C. M., D. G. McDonald, C. E. Booth, B. P. Simons, C. G. Ingersoll, and H. L. Bergman. 1988a. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. 45: 1587–1596.

Wood, C. M., B. P. Simons, D. R. Mount, and H. L. Bergman. 1988b. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 2. Blood parameters by cannulation. Can. J. Fish. Aquat. Sci. 45: 1597–1605.

Youson, J., and C. Neville. 1987. Deposition of aluminum in the gill epithelium of rainbow trout (*Salmo gairdneri* Richardson) subjected to sublethal concentrations of the metal. Can. J. Zool. 65: 647–656.

Shortening of Branchial Tight Junctions in Acid-Exposed Rainbow Trout (*Oncorhynchus mykiss*)

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The objective of this study was to investigate possible sites for Na^+ loss in fish exposed to low environmental pH. In rainbow trout (*Oncorhynchus mykiss*) exposed to pH 4.0 for 1 h, a net loss of Na^+ was stimulated, and changes in gill structure occurred. In addition to epithelial lifting and necrosis in the gills of acid-exposed fish, tight junctions between pavement epithelial cells and chloride cells decreased in length by 25% whereas tight junctions between adjacent pavement cells did not significantly change. In a second experiment where fish were moved from pH 4.0 or 3.5 water to pH 6.5 water, we observed that Na^+ loss declined immediately and approached control levels. The reversible nature of the stimulation of Na^+ loss indicates that the site of Na^+ loss in the fish gill can be reversibly opened and closed, which is consistent with the known properties of tight junctions. We hypothesize that the opening of tight junctions contributes to the loss of plasma electrolytes at low environmental pH. However, the relative magnitude of electrolyte loss through the tight junctions remains unknown.

On a tenté d'identifier les sites potentiels de perte de Na^+ chez des poissons exposés à un faible pH environnemental. Chez des truites arc-en-ciel (*Oncorhynchus mykiss*) exposées pendant 1 h à un pH 4,0, on a observé une perte nette de Na^+ et des modifications de la structure des branchies. En plus du soulèvement de l'épithélium et de la nécrose des branchies chez les poissons exposés à un milieu acide, la longueur des jonctions serrées entre les cellules épithéliales pavimenteuses et les cellules à chlorures a diminué de 25 %, tandis que la longueur des jonctions serrées entre des cellules pavimenteuses voisines n'a pas nettement varié. Lors d'une deuxième expérience où les poissons ont été transférés d'un milieu de pH 4,0 ou 3,5 à un milieu de pH 6,5, la perte de Na^+ a diminué immédiatement pour atteindre presque les niveaux témoins. La réversibilité du phénomène de stimulation de la perte de Na^+ révèle que le site de perte de Na^+ dans les branchies d'un poisson peut être ouvert et fermé de façon réversible, un fait conséquent avec les propriétés connues des jonctions serrées. Les auteurs formulent l'hypothèse que l'ouverture des jonctions serrées contribue à la perte d'électrolytes du plasma en présence d'un faible pH environnemental. Toutefois, l'importance relative de la perte d'électrolytes par ces jonctions demeure inconnue.

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The acidification of soil and water by acidic precipitation is a problem of major concern. Fish populations have been lost from many lakes and rivers of North America, Europe, and Scandinavia (Haines 1981). Similarly, naturally acid waters, such as bogs and blackwater rivers, can be the primary factor influencing the distribution and abundance of aquatic biota in many geographic regions (Rahel and Magnuson 1983; Grahm and Hastings 1984; Freda and Dunson 1986). Interest in acidification has fostered a large body of literature describing the physiological effects of acidic water on fish. The chief toxic action of soft, acidic water on fish is disruption of branchial ion regulation (see review by McDonald 1983). At low environmental pH (<5.0), active uptake of Na^+ and Cl^- is inhibited, and the diffusive loss of these ions is accelerated. The stimulation of ion loss is quantitatively responsible for net ion losses which ultimately cause the depressions in plasma Na^+ and Cl^- levels. The rate of ion loss dictates both intra- and interspecific variation in acid tolerance, and death ensues when approximately 50% of body Na^+ is lost (Freda and McDonald 1988).

The exact location for Na^+ and Cl^- loss from the fish gill during acid exposure has remained elusive. Several histological studies have demonstrated that gills of acid-exposed fish generally exhibit nonspecific indications of stress such as epithelial lifting, fusion, necrosis, and chloride cell proliferation or destruction (Daye and Garside 1976; Jagoe and Haines 1983; Leino and McCormick 1984; Leino et al. 1987; Tietge et al. 1988). The complete sloughing of the branchial epithelia that has been observed at acutely lethal pH's certainly has dire consequences for ion and water balance, as well as respiration and acid–base regulation. However, the location for ion loss at chronically lethal pH's and sublethal pH's remains unknown because damage is absent or less severe. Another possible route for ion loss is advocated by studies of isolated epithelia. Exposure to low-pH water increases the permeability of frog skin and fish opercular membranes by specifically disrupting tight junctions, thereby increasing the permeability of the paracellular pathway (Ferreira and Hill 1982; Marshall 1985).

The objectives of this study were to identify and quantify alterations in the structure of the branchial epithelium in acid-

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exposed fish and to correlate changes in structure with the magnitude of ion balance disruption. This information will point out the relative importance of the two hypothesized loss routes for ions during acid exposure, i.e. nonspecific epithelial destruction and the opening of tight junctions which seal the paracellular loss pathway.

Materials and Methods

Experimental Animals

Fingerling rainbow trout (*Oncorhynchus mykiss*) were obtained from Como Bluffs hatchery (Albany Co., Wyoming). All fish were maintained in pH 6.5 soft water for a minimum of 1 mo prior to experimentation. Soft water contained 1 mg $Na^+ \cdot L^{-1}$ and 1 mg $Ca^{2+} \cdot L^{-1}$, and its preparation is described in Mount et al. (1988). Water temperature of both holding and experimental water was 15°C and ambient lighting was used. Fish were fed commercially prepared trout chow except the day prior to experiments to reduce fouling of test water by feces. The mean ($\pm$ SD) mass of fish was 10 ± 5 g.

Flux Measurements

To measure Na^+ exchange with the water, fish were placed in 300-mL polyethylene containers. Each container had an overflow port (on the side) and three holes in the lid for an aeration line (PE 50 tubing), a water supply line (gravity fed, 100 $mL \cdot min^{-1}$), and a port for water sampling. The water supply line could be connected to one of three head tanks where soft water was continuously adjusted to either pH 6.5, 4.0, or 3.5 with H_2SO_4. The pH was monitored and adjusted continuously by a pH analyzer/controller (Leeds & Northrup model 7083). Fish were first exposed for 24 h to pH 6.5 soft water. Next, the supply lines for acid-exposed fish were switched to either the pH 4.0 or 3.5 head tank, while control fish continued to receive pH 6.5 water. Approximately 10 min after the lines were switched, the exposure water reached its target pH. After an additional 10 min of flushing, the input tubes of all the containers were removed and the overflow ports were plugged. The net flux of Na^+ of the fish was determined by measuring the change in Na^+ concentration of the water after a 1-h period. Net flux of Na^+ should approximate unidirectional efflux at pH 4.0 and 3.5 because Na^+ influx is completely inhibited at these pH's (Freda and McDonald 1988). pH drift never exceeded 0.50 and 0.25 unit at pH 4.0 and 3.5, respectively. The concentration of Na^+ in the water was determined by atomic absorption spectroscopy (Perkin-Elmer model 2380).

We conducted two series of experiments. In the first, net Na^+ flux was measured in fish exposed to pH 4.0 or 6.5 followed by histological examination of the gills. Second, to determine if the acceleration of Na^+ loss in fish is reversible over a short period of time (1 h), we first measured Na^+ loss in fish in pH 6.5 water, then in pH 4.0 or 3.5 water, and again in pH 6.5 water. No histological examinations of these fish were made.

Tissue Preparation

Following the 1-h exposure, fish were removed from test chambers, killed by a blow to the head, blotted with tissue paper, and weighed to 0.1 g. The second gill arch on the right side was immediately excised and immersed in a mixture of 2.5% gluteraldehyde and 2% tannic acid in a 0.05 M sodium phosphate buffer adjusted to pH 7.4 with NaOH. Primary fixation continued for 18 h at 4°C. After a rinse in the fixation buffer containing 6% sucrose, the tissue was postfixed at room temperature in a 1% phosphate-buffered osmium tetroxide with 3% added sucrose for 1 h. Dehydration in a series of graded ethanols and infiltration using propylene oxide as a transition solvent preceded flat embedment in Araldite 502 epoxy resin.

Sectioning

Blocks were carefully trimmed and oriented so that longitudinal sections perpendicular to the surface of the secondary lamellae were obtained to insure analogous sectioning planes. Semithin (1 μm) and thin (70 nm) sections were cut on a Porter-Blum MT-2 ultramicrotome. One-micrometer sections containing several primary filaments were mounted on glass slides and stained for 1 min with Azure B. After these sections were evaluated for orientation and qualitative changes, blocks were trimmed to smaller size and thin sections for electron microscopy were transferred to thin-bar copper grids and stained with aqueous uranyl acetate for 1 h and lead citrate for 15 min.

Light Microscopy

First, each section was evaluated qualitatively for epithelial lifting and general damage (i.e. vacuolation and sloughing) and then photographed with a Carl Zeiss photomicroscope. Next, point-count stereology methods (Weibel 1963) were used to quantify the volume density of empty lymphatic space and chloride cell numbers. Slides were examined with an Olympus BH-2 light microscope with a 10 mm square lattice grid in the eyepiece ocular which gave a regular array of 121 "points" superimposed over the section. Under oil immersion (1000×), 25 microscope fields were chosen by randomly selecting primary filaments and then selecting fields within the filament with a random number generator. Before counting each field, the eyepiece grid was rotated so that points did not fall parallel to the secondary lamellae. Points falling on (1) profiles of chloride cells in and adjacent to the secondary lamellae, (2) within empty lymphatic space created by separation of the outer epithelial layer and the pillar cell network, and (3) on all other tissue components were recorded separately using a tally counter until at least 750 points were counted. The volume density was then calculated by the equation $Vv = pI/pT \times 100$ where Vv = relative density of component, PI = number of points falling on the parameter of interest, and pT = the number of points falling on all other tissue components.

Electron Microscopy

Ultrathin sections were examined using an RCA-EMU 4 electron microscope. Sections were scanned systematically and a micrograph was taken each time a complete junctional complex (see Fig. 1) was encountered. An average of nine junctional complexes were photographed per fish. Junctions were classified into one of two types: (1) junctions between pavement epithelial cells and (2) junctions between chloride cells and pavement cells. Only a limited number of chloride – chloride cell junctions were observed; these were excluded from this analysis. Morphometric measurements of junctional complexes were made from micrographs printed at 45 120× magnification using a digitizing tablet (Houston Instruments) interfaced with an IBM-XT computer equipped with Bioquant (R and M Biometrics). Measured parameters included (1) tight

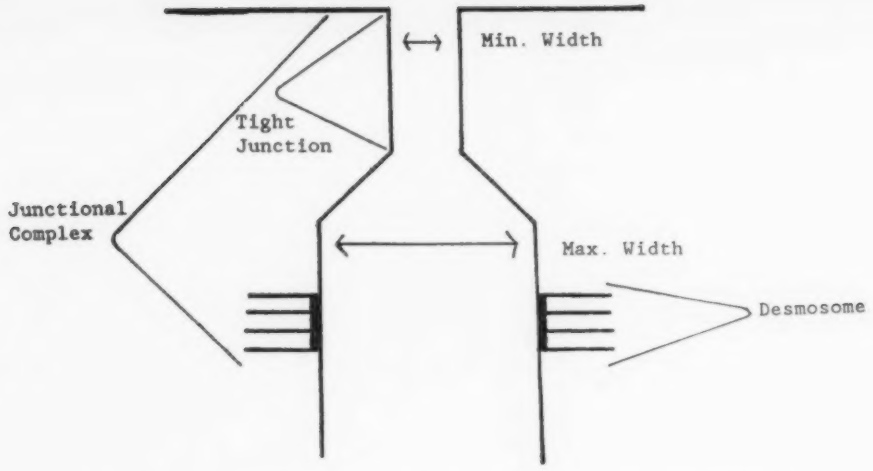

FIG. 1. Graphical illustration of a junctional complex showing all measured components.

TABLE 1. Net flux of Na^+ and the volume density of chloride cells and empty lymphatic space caused by epithelial lifting in rainbow trout exposed to either pH 6.5 or 4.0 for 1 h. Values are means ± SEM; $n = 5$ fish per treatment. An asterisk indicates a significant difference ($\alpha = 0.05$) between the two pH treatments.

Treatment	Net Na^+ flux ($\mu equiv \cdot kg^{-1} \cdot h^{-1}$)	Volume density (%)	
		Chloride cells	Lymphatic space
pH 6.5 (control)	+83 ± 26	11 ± 2	2 ± 1
pH 4.0	−1288 ± 225*	15 ± 3	12 ± 3*

TABLE 2. Net Na^+ flux in rainbow trout during 1-h exposures to pH 6.5 water, then to low-pH water (4.0 or 3.5), and then again to pH 6.5 water. All values are means ± SEM; $n = 7$ and 6 for pH 4.0 and 3.5, respectively. Values followed by the same letters are not significantly different ($\alpha = 0.05$).

Exposure pH	Net Na^+ flux ($\mu equiv \cdot kg^{-1} \cdot h^{-1}$)	
	Test pH = 4.0	Test pH = 3.5
Control (pH 6.5)	+89 ± 31 a	+19 ± 48 a
Test pH (4.0 or 3.5)	−944 ± 150 b	−4588 ± 380 b
Recovery (pH 6.5)	−301 ± 189 ac	−466 ± 64 c

junction length, (2) tight junction to desmosome length, (3) desmosome length, and (4) intramembrane distance in the tight junction (minimum) and immediately before the desmosome (maximum; see Fig. 1). The dimensions of these structures are reported as a percent of the total junctional complex to eliminate any bias caused by possible variability in sectioning angle.

Statistical Analysis

All values are reported as means ±1 standard error of the mean (SEM). Mean values for each variable were computed for each fish and these means were averaged to arrive at treatment means. Two sample *t*-test's ($\alpha = 0.05$) were used to test if means of physiological and histological variables were significantly different. All percent data were transformed using the arcsine square root transformation of Steel and Torrie (1960) before testing. Pearson's correlation coefficients were computed to determine the relationship between net Na^+ flux and histological variables.

Results

Na^+ Balance

Control rainbow trout tested in pH 6.5 soft water were in positive Na^+ balance (Table 1). When exposed to pH 4.0 water, fish were in negative Na^+ balance and were losing Na^+ at a high rate. As previously mentioned, net Na^+ loss at pH 4.0 and below is equal to unidirectional Na^+ efflux because Na^+ influx is completely inhibited (Freda and McDonald 1988).

The results of a second experiment examined the reversibility of Na^+ efflux acceleration (Table 2). Fish exposed to pH 6.5 water were in positive Na^+ balance, but when water pH was lowered to either 4.0 or 3.5, large net losses of Na^+ occurred. When fish were returned to pH 6.5 after 1 h of exposure to pH 4.0, net loss of Na^+ declined significantly. Three of the seven fish exposed to pH 4.0 returned to positive Na^+ balance during the recovery period, even though their net loss of Na^+ was high in pH 4.0 water (860 ± 55 $\mu equiv \cdot kg^{-1} \cdot h^{-1}$). Similarly, when pH 3.5 exposed fish were returned to pH 6.5 water, Na^+ loss declined significantly. No fish exposed to pH 3.5 was able to regain positive Na^+ balance during recovery.

Gill Morphology

Four of the five control fish showed normal gill morphology with little empty lymphatic space or necrosis (Table 1; Fig. 2a). However, one control fish had 6% empty lymphatic space, yet this fish had the second highest net uptake of Na^+. Fish exposed to low pH exhibited classic symptoms of acid stress. In four of five fish the gill epithelium had lifted off the pillar cell network creating large areas of empty lymphatic space (Table 1; Fig. 2b). In contrast, one fish had only 1% empty lymphatic space, yet Na^+ loss was 1149 $\mu equiv \cdot kg^{-1} \cdot L^{-1}$. Percent lymphatic space and net ion loss were not correlated in fish exposed

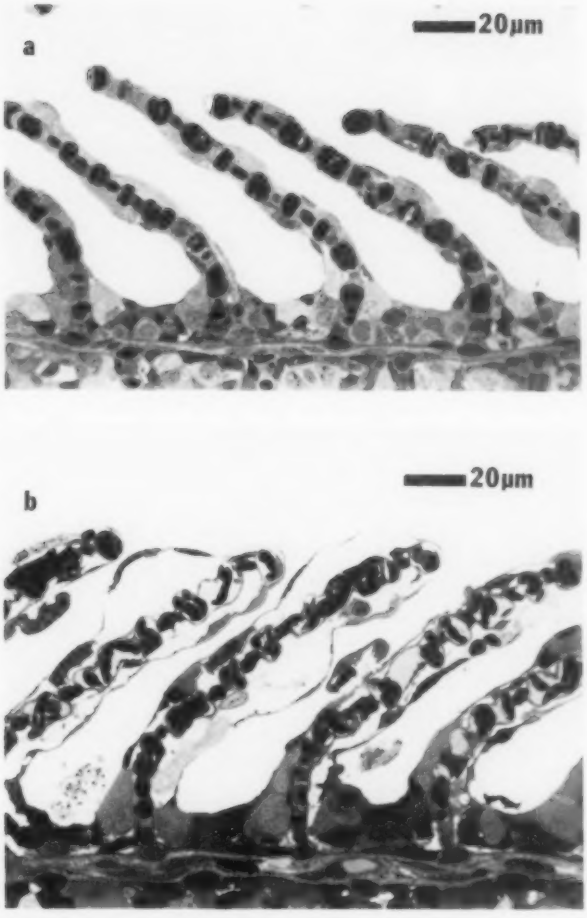

FIG. 2. Secondary lamellae of rainbow trout exposed to (a) pH 6.5 and (b) pH 4.0. Notice extensive regions of epithelial lifting in pH 4.0 exposed fish (584×).

to pH 4.0. Other damage to the gill epithelium at pH 4.0 included vacuolation, necrosis, and sloughing of pavement and chloride cells. As expected, brief acid exposure had no significant effect on the numbers of chloride cells (Table 1).

Gill Ultrastructure

We made measurements of junctional complexes and their components between pavement epithelial cells and between pavement cells and chloride cells. A micrograph of a junctional complex between two pavement cells in a control fish is shown in Fig. 3a. The length of the junctional complex and the percent occupied by the desmosome were not significantly different between fish exposed to pH 6.5 or 4.0 water in either pavement – pavement cell or pavement – chloride cell junctions (Table 3). The percent of the junctional complex that was occupied by the tight junction was not significantly different between pH 4.0 and 6.5 pavement – pavement cell junctions, but was significantly reduced by 25% at pH 4.0 in pavement – chloride cell junctions. The length of these pavement – chloride cell junctions (or its percent of the junctional complex) in low pH exposed fish was not correlated with their level of Na^+ loss.

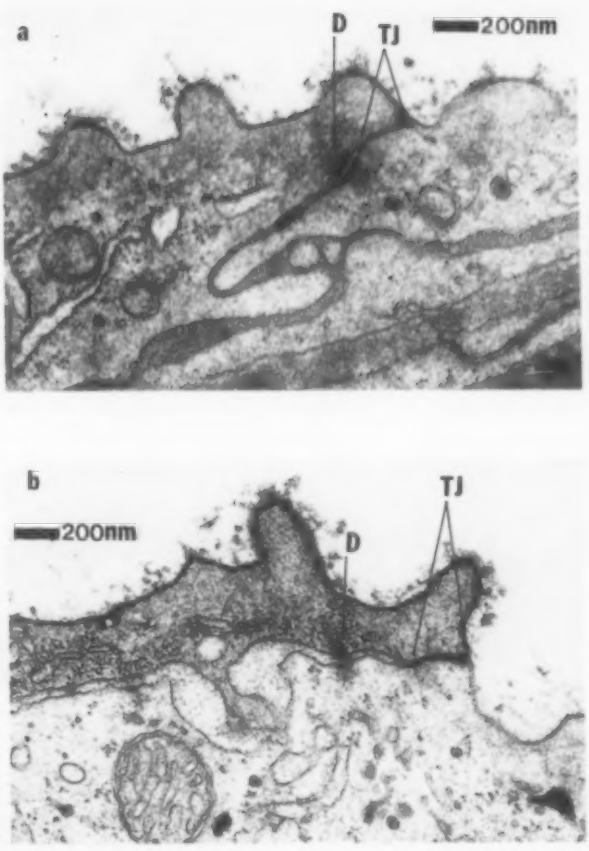

FIG. 3. Electron micrographs (44 040×) of junctional complexes in rainbow trout exposed to (a) pH 6.5 and (b) pH 4.0. Figure 3a shows a pavement – pavement junction and Fig. 3b shows a pavement – chloride cell junction. D, desmosome; TJ, tight junction.

A micrograph of a junctional complex between a pavement and a chloride cell in an acid-exposed fish is shown in Fig. 3b. In all junctional complexes examined, tight junctions were always present. Intramembrane distance was not significantly different between junction types and was not significantly affected by low pH. The mean (treatment and control) for the minimum (within tight junction) and maximum (apical side of desmosome) intramembrane distance was 12 ± 2 and 33 ± 6 nm (n = 19), respectively.

Discussion

In this study, we simultaneously examined both the histological and physiological effects of low environmental pH in intact fish after only 1 h of exposure to acidic water. Virtually all previous studies have examined changes in gill morphology after days or weeks of experimental exposure to acidic water (Leino and McCormick 1984; Karlsson-Norrgren et al. 1986a; Leino et al. 1987; Tietge et al. 1988; Mueller et al. 1991), in fish captured from acidified lakes and streams (Chevalier et al. 1985; Karlsson-Norrgren et al. 1986b), or in moribund animals (Daye and Garside 1976; Jagoe and Haines 1983). After prolonged exposure to acid water, it would be very difficult to

TABLE 3. Junctional complex length (nm) and the percent of the junction complex length occupied by the tight junction and the desmosome in rainbow trout exposed to pH 6.5 or 4.0. An asterisk indicates a significant difference ($\alpha = 0.05$) from the corresponding value in the other pH treatment. Values are means ± SEM and the number of fish examined are in parentheses. A mean of nine junctions per fish were examined of which there were approximately equal numbers of the two junction types.

	Junctions between pavement cells			Junctions between pavement and chloride cells		
Treatment	Complex length	% desmosome	% tight junction	Complex length	% desmosome	% tight junction
pH 6.5	651 ± 67 (5)	21 ± 4 (5)	64 ± 6 (5)	597 ± 161 (5)	16 ± 8 (5)	60 ± 10 (5)
pH 4.0	449 ± 82 (4)	23 ± 4 (4)	54 ± 6 (4)	580 ± 60 (5)	18 ± 5 (5)	45 ± 8* (5)

determine the primary cause for ion balance disruption because other effects such as secondary nonspecific damage or compensatory modifications may have occurred. We feel that measuring Na^+ loss during the first hour of exposure and examining the gill immediately afterward would best point out the structural changes that are responsible for increased branchial permeability.

It has been hypothesized for several years that Na^+ loss in acid-exposed fish is caused by the opening of tight junctions (McDonald 1983). This study presents the first experimental data for intact fish that tight junction structure is indeed altered by exposure to low environmental pH. Specifically, the length of tight junctions between pavement epithelial cells and chloride cells decreased by 25% after 1 h of exposure to pH 4.0. It is well documented that tight junctions are major barriers to the movement of water and ions through the paracellular pathway and are a dominant feature regulating the permeability of many epithelial tissues (see review by Schneeberger and Lynch 1984). The decrease in the length of tight junctions in this study would presumably increase the permeability of the branchial epithelium to ions via the paracellular pathway. Changes in the permeability of fish gills as a result of tight junction modification have been observed under other circumstances. When euryhaline fish are adapted to seawater, permeability of the branchial epithelia increases dramatically (Evans 1984) and is thought to arise from the shortening of tight junctions between adjacent chloride cells (Sardet et al. 1979). In addition, for acid- and seawater-exposed fish, the tight junctions appear to "unzip" from the interior to the exterior of the cell (Sardet et al. 1979). Low pH has also been shown to open tight junctions in isolated opercular membranes and frog skin (Marshall 1985; Ferreira and Hill 1982).

It was not possible to determine how much Na^+ leaked through the tight junctions at low pH because other damage occurred (epithelial lifting and cellular necrosis) which also created possible loss routes. In addition, neither tight junction length nor percent lymphatic space (caused by epithelial lifting) was correlated with Na^+ loss at pH 4.0, so the relative importance of these two loss sites remained unclear. In long-term studies, epithelial lifting and cellular necrosis were thought to be the primary effect of acid exposure, but several findings refute its importance. First, one of the control fish was in positive Na^+ balance even though it exhibited moderate epithelial lifting, and one of the acid-exposed fish which suffered high rates of Na^+ loss displayed little epithelial lifting. Second, many toxicants such as Cd and Zn cause epithelial lifting and necrosis without a concomitant increase in Na^+ loss (Spry and Wood 1985; Mallatt 1985; Reid and McDonald 1988). Third, McDonald et al. (1991) measured large net losses of Na^+ (>2000 $\mu equiv \cdot kg^{-1} \cdot L^{-1}$) from four species of fish which displayed no epithelial lifting. Lastly, when acid-exposed fish were returned to circumneutral water, net Na^+ loss was cut in half in some fish, while positive Na^+ balance was completely restored in others. It is well known that tight junction opening is immediately reversible (Schneeberger and Lynch 1984) whereas it is reasonable to expect that epithelial lifting and necrosis would require longer periods of time to heal.

In acidic water, tight junctions may have opened because surface-bound Ca^{2+} was leached from the branchial epithelium. The removal of Ca^{2+} from the bathing solution opens tight junctions and increases the permeability of frog skin (Gonzalez et al. 1978). Studies by McWilliams (1983) and Freda and McDonald (1988) have strongly suggested that low-pH water removes surface-bound Ca^{2+} from fish gills. Removal of surface-bound Ca^{2+} may potentially affect tight junctions in two ways. First, Ca^{2+} binds to fixed negative charges such as $-COO^-$, SO_4^{2-}, and PO_4^{3-} groups present on proteoglycans and glycoproteins of cell membranes (Gordon and Sauerheber 1982). Within intercellular junctions, Ca^{2+} may also form cross-links between adjacent cell membranes. If Ca^{2+} is leached from these binding sites by H^+, the integrity of the junctional complex might be compromised. Second, there is increasing evidence that Ca^{2+} may control tight junction structure by influencing the cytoskeleton which is intimately linked with the junctional complex (Schneeberger and Lynch 1984).

In summary, low environmental pH appears to open tight junctions between pavement cells and chloride cells which may promote the loss of plasma electrolytes through the paracellular pathway. However, it was not possible to determine the importance of this loss route because other damage (epithelial lifting, cellular necrosis) also occurred. Our data did indicate that epithelial lifting was not essential for electrolyte loss to occur at low pH because fish with little lifting exhibited high rates of Na^+ loss.

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References

CHEVALIER, G., L. GAUTHER, AND G. MOREAU. 1985. Histopathological and electron microscope studies of gills of brook trout, *Salvelinus fontinalis*, from acidified lakes. Can. J. Zool. 63: 2062–2070.

DAYE, P., AND E. GARSIDE. 1976. Histopathological changes in surficial tissues of brook trout, *Salvelinus fontinalis* (Mitchill), exposed to acute and chronic levels of pH. Can. J. Zool. 54: 2140–2155.

EVANS, D. H. 1984. The roles of gill permeability and transport mechanisms in euryhalinity, p. 239–282. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. X. Academic Press, New York, NY.

FERREIRA, K. T. G., AND B. S. HILL. 1982. The effect of low environmental pH on properties of the paracellular pathway and junctional structure in isolated frog skin. J. Physiol. 332: 59–67.

FREDA, J., AND W. A. DUNSON. 1986. Effects of low pH and other chemical variables on the local distribution of amphibians. Copeia 1986: 454–466.

FREDA, J., AND D. G. MCDONALD. 1988. Physiological correlates of interspecific variation in acid tolerance in fish. J. Exp. Biol. 136: 243–258.

GONZALEZ, E., T. KIRCHAUSEN, H. LINARES, AND G. WHITTEMBURY. 1978. Observations on the action of urea and other substances in opening the paracellular pathway in amphibian skins, p. 43–52. *In* K. Schmidt-Nielsen, L. Bolis, and S. H. P. Maddrell [ed.] Comparative physiology: water, ions and fluid mechanics. Cambridge University Press, Cambridge.

GORDON, L. M., AND R. D. SAUERHEBER. 1982. Calcium and membrane stability, p. 3–16. *In* L. J. Anghileri and A. M. Tuffet-Anghileri [ed.] The role of calcium in biological systems. Vol. II. CRC Press, Boca Raton, FL.

GRAHM, J. H., AND R. W. HASTINGS. 1984. Distributional patterns of sunfishes on the New Jersey coastal plain. Environ. Biol. Fishes 10: 137–148.

HAINES, T. A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Trans. Am. Fish. Soc. 110: 669–707.

JAGOE, C. H., AND T. A. HAINES. 1983. Alterations in gill epithelial morphology of yearling sunapee trout exposed to acute acid stress. Trans. Am. Fish. Soc. 112: 689–695.

KARLSSON-NORRGREN, L., I. BJORKLUND, O. LJUNGBERG, AND P. RUNN. 1986a. Acid water and aluminum exposure: experimentally induced gill lesions in brown trout, *Salmo trutta* L. J. Fish. Dis. 9: 11–25.

KARLSSON-NORRGREN, L., W. DICKSON, O. LJUNGBERG, AND P. RUNN. 1986b. Acid water and aluminum exposure: gill lesions and aluminum accumulation in farmed brown trout, *Salmo trutta* L. J. Fish Dis. 9: 1–9.

LEINO, R. L., AND J. H.MCCORMICK. 1984. Morphological and morphometrical changes in chloride cells of the gills of *Pimephales promelas* after chronic exposure to acid water. Cell Tissue Res. 236: 121–128.

LEINO, R. L., J. H. MCCORMICK, AND K. M. JENSEN. 1987. Changes in the histology of fathead minnows and yellow perch transferred to soft water or acidified soft water with particular reference to chloride cells. Cell Tissue Res. 250: 389–399.

MALLATT, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can. J. Fish. Aquat. Sci. 42: 630–648.

MARSHALL, W. S. 1985. Paracellular ion transport in trout opercular epithelium models osmoregulatory effects of acid precipitation. Can. J. Zool. 63: 1816–1822.

MCDONALD, D. G. 1983. The effects of H^+ upon the gills of freshwater fish. Can. J. Zool. 61: 691–703.

MCDONALD, D. G., J. FREDA, V. CAVDEK, R. GONZALEZ, AND S. ZIA. 1991. Interspecific differences in gill morphology of freshwater fish in relation to tolerance of low pH environments. Physiol. Zool. 64: 124–144.

MCWILLIAMS, P. G. 1983. An investigation of bound calcium from the gills of the brown trout, *Salmo trutta*, in acid media. Biochem. Physiol. 74A: 107–116.

MOUNT, D. R., C. G. INGERSOLL, D. D. GULLEY, J. D. FERNANDEZ, T. W. LAPOINT, AND H. L. BERGMAN. 1988. Effect of long-term exposure to acid, aluminum, and low calcium on adult brook trout (*Salvelinus fontinalis*). 1. Survival, growth, fecundity, and progeny survival. Can. J. Fish. Aquat. Sci. 45: 1623–1632.

MUELLER, M. E., D. A. SANCHEZ, H. L. BERGMAN, D. G. MCDONALD, R. G. RHEM, AND C. M. WOOD. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. Can. J. Fish. Aquat. Sci. 48: 2016–2027.

RAHEL, F. J., AND J. J. MAGNUSON. 1983. Low pH and the absence of fish species in naturally acidic Wisconsin Lakes: inferences for cultural acidification. Can. J. Fish. Aquat. Sci. 40: 3–9.

REID, S. D., AND D. G. MCDONALD. 1988. Effects of cadmium, copper, and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45: 244–253.

SARDET, C., M. PISM, AND J. MAETZ. 1979. The surface epithelium of teleostean fish gills. J. Cell Biol. 80: 96–117.

SCHNEEBERGER, E. E., AND R. D. LYNCH. 1984. Tight junctions, their structure, composition and function. Circ. Res. 55: 723–733.

SPRY, D. J., AND C. H. WOOD. 1985. Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural water. Can. J. Fish. Aquat. Sci. 42: 1332–1341.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York, NY.

TIETGE, J. E., R. D. JOHNSON, AND H. L. BERGMAN. 1988. Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long-term exposure to acid and aluminum. Can. J. Fish. Aquat. Sci. 45: 1643–1648.

WEIBEL, E. R. 1963. Principles and methods for the morphometric study of the lung and other organs. Lab. Invest. 12: 131–155.

LETTERS AND COMMENTS
LETTRES ET COMMENTAIRES

Corrected Euphausiid Abundance Estimates from Hydroacoustics: Comments on Cochrane et al. (1991)

Cochrane et al. (1991) recently presented a qualitative and quantitative hydroacoustic assessment of zooplankton and nekton distributions in the basins of the Nova Scotian Shelf at frequencies of 12, 50, and 200 kHz. Our qualitative conclusions are valid, but significant readjustments to our backscatter estimates, especially those at 200 kHz, are necessary: further study has revealed that the acoustic absorption coefficients used in data reduction, computed by the theory of Clay and Medwin (1977), are inaccurate in the applicable temperature ranges.

The Clay and Medwin absorption formulation at 50 and 200 kHz is dominated by the magnesium sulfate relaxation term as formulated by Schulkin and Marsh (1962). We were unaware of Fisher and Simmons' (1977) laboratory measurements at 4°C that indicated much lower absorptions between 10 and 400 kHz than predicted by the Schulkin and Marsh equation. Also, Francois and Garrison (1982a, 1982b) considered extensive accumulated field and laboratory databases to refine the absorption equations; the largest deviations from the Schulkin and Marsh relations were confined to frequencies >50 kHz and temperatures <10°C. We now consider both the Fisher and Simmons and Francois and Garrison formulations to be substantially more accurate than that published by Clay and Medwin.

Acoustic scattering levels from the deep euphausiid layers ("blue" layers) in Emerald and LaHave basins have been recomputed (Table 1). Peak S_v levels at 200 kHz calculated using the Fisher and Simmons and Francois and Garrison theories agree within 1.3 dB but fall below the Clay and Medwin theory values by roughly 6–7 dB. The S_v differentials between 200 and 50 kHz are also decreased compared to the earlier Clay and Medwin computed values. Table 1 also contains the results of inverse modelling of tabulated peak and differential S_v values using the methodology described in the previous paper: the lower 200- to 50-kHz S_v differentials require significantly larger organisms with correspondingly higher target strengths. Lower 200-kHz S_v levels combined with higher individual target strengths dictate roughly an order of magnitude reduction in euphausiid concentrations compared with the previous Clay and Medwin based results. The 28-mm mean euphausiid size observed in BIONESS catches (Cochrane et al.) appears marginally more consistent with that computed using the Francois and Garrison and Fisher and Simmons attenuations – especially the latter.

Daytime column integrated euphausiid densities using acoustics (Table 2) are significantly reduced from the previously reported 1250 and 880/m^2 for Emerald and LaHave basins, respectively. The new column densities from acoustics, nevertheless, still greatly exceed the corresponding respective column densities of 10–20 and 5/m^2 deduced from BIONESS sampling.

Appearance of the colour sections reproduced in Cochrane et al. would be only slightly altered with the new absorption coefficients. The blue primary component (200 kHz) would be most affected. Because the blue horizons are confined to a relatively narrow depth range, and square root amplitude compression and arbitrary channel amplitude scalings are applied for optimum visual presentation, the sections need change little in appearance. In Emerald Basin the midwater euphausiid swarms and lower water column blue layer would assume more identical shades of blue-green but the visual effect would be subtle.

The small differentials between 50- and 200-kHz S_v values make size estimates especially sensitive to calibration and other systematic measurement errors. These may include transducer temperature coefficients and range-dependent signal loss from tow body attitude instability — effects difficult to fully assess

TABLE 1. Acoustic inverse model results using the Stanton (1989) fluid cylinder model and assuming a Gaussian distribution of euphausiid orientations about the horizontal of 30° S.D. Data inputs consist of peak 200-kHz S_v levels and 200-versus 50-kHz S_v differentials derived using the original Clay and Medwin acoustic absorption formulation and the alternative formulations of Fisher and Simmons and Francois and Garrison, respectively. In the Fisher and Simmons case, magnesium sulfate absorption is assumed directly proportional to salinity.

Absorption model	$S_{v\,200}^{(\mathrm{Peak})}$	$S_{v\,200\text{–}50}^{(\mathrm{Diff.})}$	Length (mm)	Density (no./m^3)	Biomass density (g/m^3)
Emerald Basin					
Clay and Medwin	−58.7	10.5	15.3	240	14.0
Fisher and Simmons	−64.6	6.68	22.9	27.0	4.52
Francois and Garrison	−65.7	4.62	30.2	12.4	4.21
LaHave Basin					
Clay and Medwin	−60.9	7.38	21.1	74.2	10.1
Fisher and Simmons	−66.4	3.99	33.1	8.87	3.83
François and Garrison	−67.7	1.92	51.0	2.95	3.90

TABLE 2. Daytime column-integrated euphausiid densities and wet biomass densities at 200 kHz assuming a theoretical target strength of −77.3 dB for an "average" 28-mm euphausiid as observed with the BIONESS sampler. IBC_{200} is the depth-integrated volume backscattering coefficient computed for 200 kHz.

Basin	Int. range (m)	IBC_{200}	Column density (no./m^2)	Column biomass density (g/m^2)
Emerald	150–245	0.682×10^{-5}	366	102
LaHave	160–230	0.529×10^{-5}	283	79.2

at present. Future observations at frequencies below 50 kHz and/or observations at closer profiling ranges (a deep-towed acoustic system is now under development at the Bedford Institute of Oceanography) would help circumvent remaining measurement uncertainties and allow a more definitive test of inversion algorithms.

A second correction to Cochrane et al. concerns the discussion of Foote et al.'s (1990) published data set. An inadvertent error was made in extracting target strengths from these tables. In expression (8) of Cochrane et al. the correct value of B should be −46.8 dB rather than −48.2 dB. Using the corrected expression (8), a "geometric regime" target strength of −77.9 dB is predicted for a 28-mm euphausiid as observed in the Scotian Shelf basins. This compares with the theoretical target strength of −80.2 dB previously reported for a 28-mm euphausiid using the cylinder model, an orientation S.D. of 30°, and the physical parameters $g = 1.0357$ and $h = 1.0279$ quoted by Foote et al. This suggests that the cylinder model, as employed, may underestimate true target strengths by several decibels. Recomputation of the two-frequency inverse using 38 and 120 kHz average target strengths from the subset of Foote et al.'s tabulated target strengths representing organisms in the 30–32 mm range yields a length of 22.5 mm and a volume density of 3.9/m^3 compared with the 29.9 mm and 1.53/m^3 previously reported. Better inverse model results are obtained if a narrower distribution of orientations is assumed. Reducing the S.D. in orientations from 30 to 9° yields length 27.0 mm and volume density 1.025/m^3, close to the observed average length of 31.2 mm and implied volume density of 1/m^3. This raises the interesting possibility that undisturbed euphausiids may orient themselves more closely to the horizontal than previous camera studies have indicated, the presence of a camera possibly disturbing the natural distribution of orientations. More definitive data sets and refined studies of euphausiid orientation are required to answer these questions and thereby to facilitate more confident acoustic inverse estimates — N. A. Cochrane, *Department of Fisheries and Oceans, Physical and Chemical Sciences, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2, Canada*, and D. Sameoto, *Department of Fisheries and Oceans, Biological Sciences, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2, Canada.* (JB024)

References

CLAY, C. S., AND H. MEDWIN. 1977. Acoustical oceanography. John Wiley & Sons, New York, NY. 544 p.

COCHRANE, N. A., D. SAMEOTO, A. W. HERMAN, AND J. NEILSON. 1991. Multiple-frequency acoustic backscattering and zooplankton aggregations in the inner Scotian Shelf basins. Can. J. Fish. Aquat. Sci. 48: 340–355.

FISHER, F. H., AND V. P. SIMMONS. 1977. Sound absorption in sea water. J. Acoust. Soc. Am. 62(3): 558–564.

FOOTE, K. G., I. EVERSON, J. L. WATKINS, AND D. G. BONE. 1990. Target strengths of Antarctic krill (*Euphausia superba*) at 38 and 120 kHz. J. Acoust. Soc. Am. 87(1): 16–24.

FRANCOIS, R. E., AND G. R. GARRISON. 1982a. Sound absorption based on ocean measurements: Part I: Pure water and magnesium sulfate contributions. J. Acoust. Soc. Am. 72(3): 896–907.

1982b. Sound absorption based on ocean measurements: Part II: Boric acid contribution and equation for total absorption. J. Acoust. Soc. Am. 72(6): 1879–1890.

SCHULKIN, M., AND H. W. MARSH. 1962. Sound absorption in seawater. J. Acoust. Soc. Am. 34(6): 864–865.

STANTON, T. K. 1989. Simple approximate formulas for backscattering of sound by spherical and elongated objects. J. Acoust. Soc. Am. 86(4): 1499–1510.

OBITUARY/NÉCROLOGIE

Michael Waldichuk

1923–1991

Canada and the world scientific community has lost an outstanding scientist who resided and worked in British Columbia, Canada. Dr. Michael Waldichuk died unexpectedly on May 4, 1991. Up until his death, he worked as a senior scientist at the Department of Fisheries and Oceans West Vancouver laboratory. Michael was recognized worldwide as a noted authority on environmental matters, particularly contaminants and pollution. He was instrumental in founding and building the Department's laboratory in West Vancouver, the Pacific Environment Institute, in 1970. Through Michael's leadership, as Program Head, the laboratory flourished, attracting highly competent and productive scientists who have made major contributions to environmental issues and fisheries and habitat science.

Dr. Waldichuk received a B.A. Honors in Chemistry in 1948 and an M.A. in 1950 from the University of British Columbia and a Ph.D. in Oceanography in 1955 from the University of Washington. His Ph.D. thesis dealt with the physical oceanography of the Strait of Georgia, British Columbia. He was hired by the Fisheries Research Board of Canada in 1952 as Associate Scientist and worked for many years at the Pacific Biological Station at Nanaimo, B.C., where he became Oceanographer-in-Charge of the Pacific Oceanographic Group in 1966. He worked extensively on pollution problems along the British Columbia coast, particularly those related to coastal pulp mills and effluent disposal problems.

Michael had an exceptional publication record, numbering in excess of 150 primary publications and approximately 100 secondary publications. His work included methodology for evaluation of effects of pollutants on marine organisms, design of outfalls in relation to oceanographic characteristics, studies of oxygen diffusion in still seawater, organic waste disposal, heavy metals pollution, radioactive waste disposal considerations, extensive studies of pulp mill waste disposal and its effects on the aquatic environment, and oceanographic surveys of British Columbia inlets and channels. He published a number of critical reviews, assessment documents, book chapters, and other significant interpretive contributions. In addition, he played a major role in investigating and advising on a variety of important and highly visible West Coast issues including disposal of mine tailings in Rupert Inlet and the Roberts Bank port expansion and edited a series of comprehensive reviews on key British Columbia estuaries. He was an editor of the *Marine Pollution Bulletin* and regularly reviewed papers for journals such as *Marine Chemistry, Water, Air and Soil Pollution, Canadian Journal of Fisheries and Aquatic Sciences*, and others.

In 1969, Dr. Waldichuk was appointed Program Head of the Pacific Environment Institute (now called the West Vancouver Laboratory) in West Vancouver, B.C., having acquired the site of the former Millerd Cannery on the waterfront near Point Atkinson. The site was selected because of the quality of its water, suitable for raising all life stages of Pacific salmon and other species. Today, the laboratory is a centre of excellence in habitat, aquaculture, fish production, biotechnology, and contaminants research. Many of the scientific staff hired by Dr. Waldichuk have gone on to make significant contributions in their fields. His enthusiasm and tireless efforts in establishing and leading the laboratory will be long remembered!

Dr. Waldichuk served as a member of the Pacific Coast Working Group on Low-Level radioactive Waste Disposal of the U.S. National Academy of Sciences, Committee on Oceanography, 1958–62; Pacific Subcommittee on Oceanography, 1967–77 (Chairman, 1968–70); Estuary Working Group, Pacific, 1971–81; the Panel on Marine Aquatic Life and Wildlife of the Committee on Water Quality Criteria, Environmental Studies Board, National Academy of Sciences - National Academy of Engineering, Washington, DC, 1971–72; the National Science Foundation *Ad Hoc* Subcommittee on Riverine Flux to the Oceans (RIVFLO) of the Advisory Committee for Ocean Sciences, Washington, DC, 1978; and the Regional Ocean Dumping Advisory Committee (RODAC), Pacific Region, administering the *Ocean Dumping Control Act*, 1976–91. He was a member of various professional societies on marine sciences and technology, including Sigma Xi, and a Fellow of the Chemical Institute of Canada.

Michael was a member of the IMCO/FAO/UNESCO/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP), 1969–77 and 1984, serving as its Chairman from 1970 to 1973 and later chairing its Working Group on the Principles of Developing Coastal Water Quality Criteria. He also was active on two GESAMP working groups dealing with interchange of pollutants between the atmosphere and the ocean and integrated global ocean monitoring, respectively. Also in the international sphere, Dr. Waldichuk was a technical advisor to the Canadian delegation at London Dumping Convention (LDC) negotiations in 1972 and Chairman of the LDC *Ad Hoc* Scientific Group on Dumping, 1978–79. He also served as technical advisor to the Canadian delegation in 1983 at a conference hosted by the Intergovernmental Maritime Consultative Organization to develop the International Convention for the Prevention of Pollution from Ships. Michael was a UNESCO nominee to the Intergovernmental Oceanographic Commission's International Coordination Group on Global Investigation of Pollution in the Marine Environment, 1974–76, chairing its Second Session in New York in July 1974 and its Third Session in Paris in 1975. Michael lectured and gave papers in a wide variety of locations around the globe and participated vigorously in many workshops, symposia, and study groups.

Dr. Waldichuk was Director of the Canadian Association for Water Pollution Research and Control (1967–68, 1971–76) and received a service award from the organization in 1988. He chaired the National Research Council of Canada's Committee on Marine Analytic Chemistry from 1985 to 1989 and was a longtime member of the British Columbia Research Council. Michael was Chairman of the Canadian National Committee for the Scientific Council on Ocean Research (SCOR) from 1989 until his death. He helped prepare the 1991 Environment Canada document "Health of our Oceans: A Status Report on Canadian Marine Environmental Quality," focussing on the situation on the West Coast of Canada.

As well as making an outstanding contribution to regional, national, and international science, Dr. Waldichuk was a very active member of the Board of Governors of the Vancouver Public Aquarium Association. He served on the Board since 1972 and was Vice President of the Association from 1975 to 1989. Up until the time of his death, Dr. Waldichuk headed the Aquarium's Research and Conservation Committee which plays an active role in all aspects of research carried out and sponsored by the Aquarium.

Dr. Waldichuk's colleagues and co-workers, his international scientific associates, his friends and colleagues from the aquarium, and the neighbors all around the West Vancouver Laboratory will long remember Mike as an outstanding scientist, as a strong and engaging personality, and as a friend. He was remarkable for his patience, dedication, and enthusiasm for science and for his interest in supporting the work of others and in seeing them progress in their work and careers.

His colleagues in GESAMP expressed the feelings of many when they recently said "He was respected as someone who could always be relied upon to meet difficult challenges with thorough, timely and thought-provoking positions and papers. Michael was also respected as a gentle, kindly and sympathetic individual who always maintained his calm and gentlemanly attitude even under difficult circumstances."

Michael Waldichuk is survived by his wife Shirley, by two sons, Tom and Andrew, and by his brother John and John's wife Andrea. A commemorative plaque has been placed near the seashore at the West Vancouver laboratory acknowledging Michael's contributions and the warm memories we have of this outstanding Canadian scientist, colleague, and friend.

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